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# AN EMPIRICAL LAW DESCRIBING HETEROGENEITY IN THE YIELDS OF AGRICULTURAL CROPS

BY H. FAIRFIELD SMITH[1]

(With Seven Text-figures)

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## 1. INTRODUCTION AND REVIEW OF LITERATURE

IN seeking to improve the efficiency of field experiments the best size of plot has been the subject of much discussion. To determine this point many experiment stations have conducted "blank experiments" (sometimes called "uniformity trials"), in which the produce from an area of ground is harvested as a number of small plots. By combining data for adjacent units the yields from plots of different sizes and shapes can be determined and their variabilities compared. Although on some points the results from these experiments have been gratifyingly consistent, no method of determining from these experiments the best size of plot for any particular purpose has heretofore been suggested. Indeed, both on this and on other points the usual methods of presenting the results of blank experiments are often misleading.

[1] Formerly Assistant Research Officer, Council for Scientific and Industrial Research, Canberra, Australia.

The usual method of evaluating the best size of plot for a given set of conditions is to refer to a figure like Fig. 2 A (p. 5) which presents data for a blank experiment with wheat using unit plots of ½ sq. ft. The argument then is: from one to about four times the unit area reduction in variability for increasing plot size is rapid. For plot sizes greater than six times the unit there is little reduction in variability. Consequently the optimum size is somewhere in the region of four to six times the unit area.

This argument fails to observe the condition that the region of maximum curvature depends entirely on the scale of the co-ordinates against which the observations have been plotted. In Fig. 2 B the ordinate scale has been doubled and the abscissa scale has been reduced to one-sixth. It gives the curve which one might ordinarily have drawn if the unit plot had been 3 sq. ft. ($x=6$) instead of ½ sq. ft. The conclusion would now be that the optimum size is somewhere in the region of 24–36 units. It will be shown that the relative rate of reduction in variability is the same for the whole range of plot sizes explored, and that consequently the above argument is fallacious.

An unscientific approach is again usually adopted when comparing the reduction of variability due to increasing size of plot with the reduction which is to be expected if the fertilities of adjacent areas of ground were uncorrelated, or which is attainable by random replication of unit plots. The latter condition is represented by the dotted lines in Figs. 2 and 3. This curve is useful for comparison, but since an hypothesis of zero correlation between adjacent areas cannot be regarded as probable (as was demonstrated by Harris, 1915, 1920) it is misleading to speak of it as "theoretically expected". Confusion thus raised seems to have led a recent writer, Wiebe (1935), into an opposite error. Recognizing that the disagreement of observed and so-called theoretical curves is due to correlation, he has calculated a correlation coefficient for each plot size and thence calculated back the "theoretical coefficient of variation when $r \neq 0$". He has not, however, calculated any theoretical curve in the algebraic sense of a locus of points conforming to some law, and the agreement between observed and calculated values tells nothing except that the arithmetic has been correct.

The usual method of representing the heterogeneity of a piece of ground is to construct a fertility contour map after the fashion of Fig. 1. This particular figure has been constructed from the yields of square plots, 2 × 2 ft., centred at distances of 1 ft. (i.e. adjacent ½ sq. ft. units were combined to show "moving averages"). But in the great majority

of blank experiments unit plots have been long and narrow, and heterogeneity maps have been constructed from such units. For comparison with these, other maps were constructed from the yields of oblong plots orientated in both directions across the field described in § 2. The contour lines thus obtained were found to run predominantly in the direction of the length of plot, whatever that direction might be. This was so because the long narrow plots failed to provide sufficient points showing where contours might be connected across the lengths of plots. This leads to an appearance of greater variability across the plots than along them, an appearance which may often be, as in the present instance, wholly fictitious.

No satisfactory quantitative measure of soil heterogeneity has heretofore been devised. Harris (1915) proposed using the intraclass correlation coefficient of yields from adjacent areas as a "coefficient of heterogeneity". But although numerous workers have taken the trouble to evaluate such coefficients for their data it does not appear to serve any other purpose than to demonstrate that the fertilities of adjacent areas are correlated. This is demonstrated equally well, if not indeed better, and with much less labour, by a figure such as Fig. 2.

A number of blank experiments have shown that in some fields the variation of fertility is greater in one direction than in another. In such fields the shape of plot requires to be considered, since plots having their lengths in the direction of greater soil variability are less variable than similar plots orientated in a direction of lesser variability. In such fields the arrangement of differently shaped plots to form blocks is critical, and some apparently anomalous results reported in the literature can be ascribed to the way in which the data have been grouped in arbitrarily arranged blocks.

With regard to shape of plot as an independent factor affecting variability the most extensive discussion has been given by Christidis (1931). Following a theoretical discussion corroborated by a certain amount of observational data this author concluded that "in no case can square plots be more uniform than long narrow ones". Unfortunately the premises whence this deduction emanates contain a limitation to the arrangement of plots within blocks which is not consonant with practice and which appears to have been overlooked. It can be shown also from published data, for exampl., Day (1920); Wood & Stratton (1909), that long narrow plots may be more or less variable than square ones depending upon their orientation. Nevertheless, it does appear that long narrow plots tend to be on the average less variable than square

plots, a finding which agrees with the observation that adjacent areas tend to be more closely correlated than more distant areas.

## 2. A BLANK EXPERIMENT WITH SMALL PLOTS OF WHEAT

Results of previous blank experiments have consistently shown that for a given area of ground maximum accuracy can be attained by the use of the smallest plots that are consistent with other requirements. In analytical yield experiments much of the labour is directly proportional to the "test area", and this may, if required, be reduced to the area occupied by a single plant per plot. In such extreme circumstances

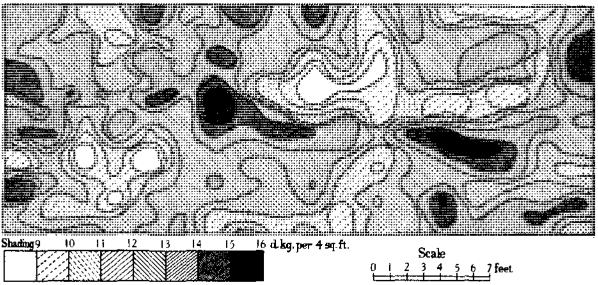


Fig. 1. Fertility contour map (based on moving averages for yield of grain from areas 2 ft. sq.)

the ratio of guard to test area will be unfavourable but may be counterbalanced if the gain in efficient use of the test area is sufficiently great. Nevertheless, a single plant per plot is unlikely to be efficient, and to obtain information as to the lower limit of plot size for which the above principle might apply a small blank experiment was conducted at Canberra in 1934.

*Description of the experiment.* Wheat of the variety Waratah was sown with a Woodfield dibber[1] which deposited the seeds uniformly 2 in. deep and 2 in. apart in rows 6 in. apart. At harvest (December 1934) four rows at each side and 1 ft. at each end of a row were discarded to avoid border effects. The remainder—15 ft. (thirty rows) by 36 ft.—was

[1] The Woodfield dihber was invented and is manufactured by C. E. Woodfield at the New Zealand Wheat Research Institute, Christchurch. It has been described in the First Annual Report of the Institute (1930), p. 6.

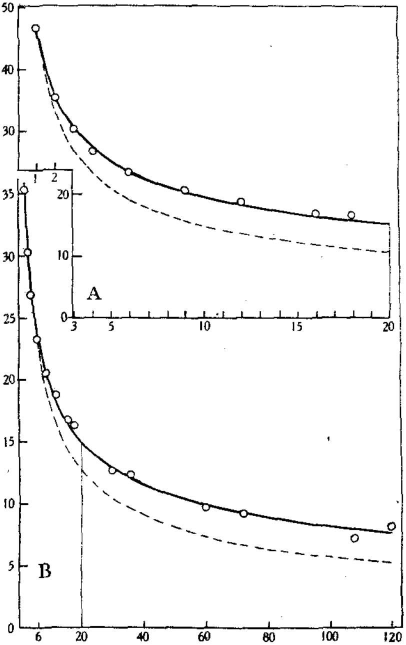


Fig. 2. Plot size and standard deviation per plot. Ordinate: standard deviation per plot ($s_x$) in decigrams per $\frac{1}{2}$ sq. ft. Abscissa: size of plot ($x$) in units of $\frac{1}{2}$ sq. ft. The dotted line indicates the reduction in standard deviation obtainable by combining units (or in $2B$ groups of 6 units) which are not correlated. The solid line represents the equation

$$s_x = \sqrt{(2137/x^{0.748})}.$$

Each point represents the average variance of all shapes of each size which could be fitted into the total area (Table II).

harvested as 1080 plots of ½ sq. ft. (1 ft. length of row).[1] The average number of plants per plot was 5·02 (from six seeds sown).

The full data for yield of grain and for numbers of ears per foot length will be lodged in the archives at the Natural History Museum at South Kensington and in the Division of Plant Industry at Canberra.

The soil heterogeneity of the area was found to be patchy and equally variable in all directions. This is shown by a fertility contour map (Fig. 1) constructed from the moving averages of plots 2 ft. sq., and is corroborated by the mean squares between columns and between pairs of rows (taken in pairs to give a width of 1 ft. and so be comparable with the columns) being similar, namely 4739 and 4560.

The distribution of yields from unit plots was symmetrical and differed from a normal distribution only very slightly in the direction of a flat-topped curve[2]—$g_2$ negative but small (Fisher, 1932, § 14).

Variances of plots of several sizes and shapes are given in Table I.

*Shape of plot.* Table I shows that there was no consistent change of variability relative to shape of plot. For any particular size the differences are nowhere statistically significant.

*Size of plot.* Fig. 2 shows that the reduction of variability with increasing plot size is similar to that which has been observed in all blank experiments previously reported, and that such reduction is less than could be obtained by equivalent random replication.

The regression of variability on plot size can be more easily interpreted when the observations are plotted on double logarithmic paper (Fig. 3). It becomes, in these conditions, linear. This means that the *relative* reduction in variability for a *relative* increase of plot size is similar throughout the range observed, that, say, doubling the plot size always results, on the average, in the same proportional reduction in variability.

The relationship can be described by an equation of the form

$$\log V_x = \log V_1 - b' \log x,$$

where $V_x$ is the variance of yield per unit area for plots of $x$ units of

[1] This arrangement was not ideal. Analysis of results for shape of plot would have been considerably simplified if unit plots had been square. Further, to facilitate the formation of numerous shapes and sizes of plots while still covering the same total area of ground, the numbers of unit plots in each direction should be a multiple of 12. These observations must have been made by all workers who have had occasion to analyse one of these experiments, but it is a practical detail which is nearly always overlooked in the preliminary planning.

[2] This was determined graphically using probability graph paper described by K. G. Karsten, *Charts and Graphs*, chap. 40. New York: Prentice-Hall Inc., 1925.

Table I. *Variances of plots of different sizes and shapes, Canberra*, 1934

| Plot size ($x$) in units of ½ sq. ft. | Plot shape: length in ft. Across rows | Along rows | Variance ($V_x$) for yield per sq. ft. in decigrams | Degrees of freedom | Area used Rows-feet (total area = 30·36) |
|---|---|---|---|---|---|
| 1 | ½ | 1 | 2201 | 1079 | Total |
| 2 | ½ | 2 | 1220 | 539 | ,, |
| | 1 | 1 | 1272 | 539 | ,, |
| 3 | ½ | 3 | 863 | 359 | ,, |
| | 1½ | 1 | 976 | 359 | ,, |
| 4 | 1 | 2 | 721 | 269 | ,, |
| 6 | ½ | 6 | 524 | 179 | ,, |
| | 1 | 3 | 523 | 179 | ,, |
| | 1½ | 2 | 582 | 179 | ,, |
| | 3 | 1 | 562 | 179 | ,, |
| 9 | ½ | 9 | 413 | 119 | ,, |
| | 1½ | 3 | 426 | 119 | ,, |
| | 4½ | 1 | 450 | 29 | 27·10 |
| 12 | ½ | 12 | 334 | 89 | Total |
| | 1 | 6 | 383 | 89 | ,, |
| | 1½ | 4 | 386 | 89 | ,, |
| | 2 | 3 | 326 | 83 | 28·36 |
| | 3 | 2 | 310 | 89 | Total |
| | 6 | 1 | 376 | 71 | 24·36 |
| 15 | ½ | 15 | 404 | 29 | 30·15 |
| 16 | 2 | 4 | 281 | 62 | 28·36 |
| 18 | ½ | 18 | 276 | 59 | Total |
| | 1 | 9 | 272 | 59 | ,, |
| | 1½ | 6 | 282 | 59 | ,, |
| | 3 | 3 | 241 | 59 | ,, |
| | 4½ | 2 | 214 | 53 | 27·36 |
| | 9 | 1 | 275 | 35 | 18·36 |
| 30 | 15 | 1 | 158 | 35 | Total |
| 36 | ½ | 36 | 102 | 29 | ,, |
| | 1 | 18 | 178 | 29 | ,, |
| | 1½ | 12 | 181 | 29 | ,, |
| | 2 | 9 | 171 | 27 | 28·36 |
| | 3 | 6 | 155 | 29 | Total |
| | 4½ | 4 | 159 | 26 | 27·36 |
| | 6 | 3 | 146 | 23 | 24·36 |
| | 9 | 2 | 167 | 17 | 18·36 |
| 60 | 2½ | 12 | 98 | 17 | Total |
| | 5 | 6 | 77 | 17 | ,, |
| | 15 | 2 | 102 | 17 | ,, |
| 72 | 1 | 36 | 63 | 14 | ,, |
| | 3 | 12 | 102 | 14 | ,, |
| | 6 | 6 | 96 | 14 | 24·36 |
| 108 | 1½ | 36 | 52 | 9 | Total |
| 120 | 5 | 12 | 32* | 8 | ,, |
| | 15 | 4 | 98* | 8 | ,, |

* Difference is not significant. $z = 0{\cdot}550 \pm 0{\cdot}353$.

area. Since the variances have been estimated from varying numbers of plots it is desirable that, when fitting a regression, each point should be weighted inversely as its variance.[1] In fitting a linear regression to these

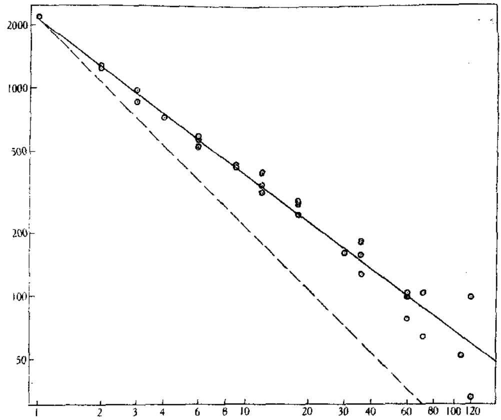


Fig. 3. Logarithmic relationship between variance per plot and plot size. Ordinate: logarithm of variance per plot ($\log s_x^2$). Abscissa: logarithm of size of plot ($\log x$). Dotted line as in Fig. 2 (but passing through the value for $s_1^2$ estimated from the regression instead of through the observed value). (Only plot sizes which fit exactly into the total area are shown.)

data the observations of the dependent variates are logarithms of variances. To a first approximation the variance of the logarithm of a variance may be taken as

$$\{\delta\,(\log_e s^2)\}^2 = \left\{\frac{2s\,\delta s}{s^2}\right\}^2 = \frac{2}{n},$$

where $\delta s = s/\sqrt{(2n)}$ represents the standard deviation of $s$ which may be considered small relative to the mean of $s$, $s^2 = V$, and $n$ is the number of degrees of freedom upon which the estimate of variance is based.

[1] Statistical methods of curve fitting rest on the assumption that observations are independent. It is clear that this condition is not fully satisfied in the present case, but all we require is a measure of an empirical relation and tests of significance are of only secondary interest.

Weighting the observations, then, inversely in proportion to $n$ the regression was found to be

$$\log_{10} V_x = 3{\cdot}3298 - 0{\cdot}7486 \log_{10} x,$$

or

$$V_x = \frac{2137}{x^{0\cdot749}},$$

where $s_x$ or $\sqrt{V_x}$ is in decigrams per $\frac{1}{2}$ sq. ft., and $x$ is in units of $\frac{1}{2}$ sq. ft. The apparent[1] standard error of the $b'$ coefficient (0·7486) is $\pm 0{\cdot}0132$.

### 3. Review of blank experiments previously reported

In order to determine whether the equation representing the relationship between plot size and variability as observed in the Canberra blank experiment might be generally applicable other published data have been examined. Table II and Fig. 4 show the results for thirty-nine experiments. Excepting only a small number which had too few plots to give reliable results, or in which data have not been presented in a form adaptable for our purpose, Fig. 4 includes results of all published work available in Canberra libraries. Straight regression lines have been drawn in freehand, except that for WA and P the best fitting lines have been calculated.

It is clear that in general the regression of the logarithm of standard deviation on size of plot is substantially linear. Fields P, WA and WB show a tendency to curve upwards. For each of four experiments with sweet potatoes (FA to FH) two regressions are shown—one for single row (broken line) and one for multiple row plots. Since, however, the author (Thompson, p. 395) gives reasons for expecting that single-row plots should be more variable, and that parts of the same row may be correlated owing to peculiarities of technique with sweet potatoes, this has presumably nothing to do with soil heterogeneity. In fields HB, L, U, X and Y the results are affected by shape of plot, indicating that the soil is more variable in one direction than in another.

The values of the regression coefficients observed will be discussed in a later section after attention has been given to some theoretical matters. The extent to which variation of the coefficients may be due to differences in crop, soil or season cannot be satisfactorily determined from present data. Since errors of observation tend to lower correlation and the regression coefficient is a function of the correlation of adjacent areas, errors in technique—sowing, harvesting, threshing, weighing—and genetic variability of plants will tend to raise the observed value of $b'$

Table II. *Review of blank experiments giving estimates of* b *coefficients of heterogeneity* (*see Fig.* 4)

| Crop | Location | Size of unit plots, sq. ft. | No. of unit plots in experiment | c.v. for plots of 1/40 acre | Mean yield cwt./acre | $b'$ | $b$ | Author | Journal reference | Reference, Fig. 4 | Reference in Cochran's catalogue |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Wheat | Victoria | 272 | 160 | 5·9 | 12·2 | 0·44 | 0·33 | Forster & Vasey | *Proc. roy. Soc. Vict.* (1928), **40**, 70 | A | 119 |
| | Rothamsted | 87 | 500 | 6·3 | 17·7 | 0·46 | 0·37 | Mercer & Hall | *J. agric. Sci.* (1911), **4**, 107 | B | 124 |
| | ,, | 0·82 | 1092 | 4·4 | 19·7 | 0·54 | 0·51 | Kalamkar | *J. agric. Sci.* (1932), **22**, 783 | — | 123 |
| | Nebraska | 30 | 224 | 4·9 | 19·4 | 0·54 | 0·49 | Montgomery | *Bull. U.S. Dep. Agric.* (1913), **269** | C | 126 |
| | Missouri | 3·3 | 3100 | {3·7<br>6·9 | 11·5<br>11·5 | 0·80<br>0·58 | 0·80<br>0·47} | Day | *J. Amer. Soc. Agron.* (1920), **12**, 100 | U | 118 |
| | Australia | 0·5 | 1080 | 1·7 | 25·9 | 0·74 | 0·72 | Smith | This paper, Fig. 3 | — | 130 |
| | ,, | 2·3 | 54 | 3·1 | 21·5 | 0·54 | 0·44 | ,, | Unpublished, *Ab*, Fig. 5 | — | — |
| Wheat irr. | Idaho | 15 | 1440 | 10·5 | 33·6 | 0·22 | 0·08 | Wiebe | *J. agric. Res.* (1935), **50**, 331 | Z | 132 |
| Maize | Arkansas | 242 | 432 | 14·2 | — | 0·21 | 0·05 | McClelland | *J. Amer. Soc. Agron.* (1926), **18**, 819 | V | 23 |
| Sorghum for. | Texas | 55 | 1920 | 7·9 | 80·5 | 0·42 | 0·35 | Stephens & Vinall | *J. agric. Res.* (1928), **37**, 629 | S | 91 |
| Mangolds | Rothamsted | 218 | 200 | 4·1 | 586 | 0·48 | 0·39 | Mercer & Hall | *J. agric. Sci.* (1911), **4**, 107 | D | 43 |
| Beets | Minnesota | 61 | 600 | 5·7 | 326 | — | 0·50 | Immer | *J. agric. Res.* (1932), **44**, 649 | WA | 98 |
| | ,, | 61 | 600 | 3·8 | 323 | — | 0·74 | Immer & Raleigh | *J. agric. Res.* (1933), **47**, 591 | WB | 99 |
| Potatoes | W. Virginia | 35 | 290 | 18·9 | 84·0 | 0·29 | 0·18 | Westover | *Bull. W. Va Dep. Agric.* (1924), **189** | EA | 76 |
| | ,, | 35 | 186 | 10·5 | 116·7 | 0·45 | 0·37 | ,, | ,, ,, | EB | 76 |
| | ,, | 35 | 192 | 16·8 | 58·3 | 0·46 | 0·39 | ,, | ,, ,, | EC | 76 |
| | ,, | 35 | 258 | 16·4 | 91·4 | 0·46 | 0·40 | ,, | ,, ,, | ED | 76 |
| | ,, | 35 | 3300 | 25·1 | 56·4 | 0·32 | 0·26 | ,, | ,, ,, | EE | 76 |
| | Saskatchewan | 66 | 576 | — | — | — | 0·71 | Kalamkar | *J. agric. Sci.* (1932), **22**, 373 | X | 72 |
| | Ormskirk | 73 | 618 | — | — | — | 0·59 | Justensen | *J. agric. Sci.* (1932), **22**, 366 | Y | 71 |
| Sweet potatoes | Maryland, 1929 | 45 | 1000 | 8·6 | 41·6 | 0·78 | 0·77 | Thompson | *J. agric. Res.* (1934), **48**, 379 | FA | 75 |
| | Maryland, 1930 | 45 | 2000 | 7·0 | 102·7 | 0·66 | 0·65 | ,, | ,, ,, | FB | 75 |
| | South Carolina, 1929 | 45 | 1560 | 11·1 | 69·6 | 0·58 | 0·56 | ,, | ,, ,, | FC | 75 |
| | South Carolina, 1930 | 45 | 1035 | 10·7 | 72·3 | 0·74 | 0·73 | ,, | ,, ,, | FD | 75 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cotton irr.** | **Sakha, 1921** | 1131 | 160 | 10·5 | — | 0·32 | 0·19 | Bailey & Trought | *Bull. Minist. Agric. Egypt*, Tech. Sci. Serv. (1926), **63** | GA | 25 |
| | **Gemmeiza, 1921** | 1131 | 160 | 12·6 | — | 0·16 | ? | ,, | ,, ,, | GB | 25 |
| | **Gemmeiza, 1922** | 1131 | 160 | 9·1 | — | 0·58 | 0·53 | ,, | ,, ,, | GC | 25 |
| | **Giza, 1921** | 452 | 154 | 25·2 | — | 0·34 | 0·21 | ,, | ,, ,, | GD | 25 |
| | **Giza, 1923** | 452 | 160 | 15·0 | — | 0·47 | 0·40 | ,, | ,, ,, | GE | 25 |
| **Cotton** | **Texas (Col. St.)** | 144 | 288 | 28·9 | — | — | 0·53 | Reynolds *et al.* | *J. Amer. Soc. Agron.* (1934), **26**, 725 | HA | 32 |
| | **Texas (Chill.)** | 157 | 288 | — | — | — | 0·56 | ,, | ,, ,, | HB | 32 |
| **Soybeans for.** | **W. Virginia, 1925** | 20 | 1008 | 9·6 | 30·3 | 0·24 | 0·11 | Odland & Garber | *J. Amer. Soc. Agron.* (1928), **20**, 93 | IA | 95 |
| **Soybeans seed** | **W. Virginia, 1926** | 20 | 1540 | 6·0 | 10·7 | 0·40 | 0·34 | ,, | ,, ,, | IB | 95 |
| **Pineapples** | **Hawaii (K 19)** | — | 24 | — | — | 0·42 | 0·20 | Magistad & Farden | *J. Amer. Soc. Agron.* (1934), **26**, 631 | KA | 69 |
| | **Hawaii (K 1)** | — | 64 | — | — | 0·47 | 0·36 | ,, | ,, ,, | KB | 69 |
| **Natural pasture** | **Australia** | 22 | 720 | { 9·5<br>{ 15·8 | 8·7<br>8·7 | 0·63<br>0·37 | 0·60<br>0·29 | Davies | *Bull. Coun. sci. industr. Res. Aust.* (1931), **48** | L | 67 |
| **Oranges irr.** | **Riverside** | 3840 | 195 | 11·0 | 102 | 0·41 | 0·31 | Parker & Batchelor | *Hilgardia* (1932), **7**, 81 | M | 65 |
| | **Arlington** | 484 | 1000 | 34·3 | 110 | 0·24 | 0·10 | Batchelor & Reed | *J. agric. Res.* (1918), **12**, 245 | NA | 64 |
| | **Antelope H.** | 484 | 495 | 25·6 | 150 | 0·36 | 0·25 | ,, | ,, ,, | NB | 64 |
| | **Villa Park** | 484 | 240 | 79·8 | 198 | 0·44 | 0·32 | ,, | ,, ,, | NC | 64 |
| **Lemons irr.** | **Upland** | 576 | 364 | 26·2 | 183 | 0·43 | 0·34 | ,, | ,, ,, | O | 36 |
| **Walnuts irr.** | **Whittier** | 2500 | 280 | 57·5 | 13·5 | 0·52 | 0·47 | ,, | ,, ,, | P | 115 |
| **Apples irr.** | **Utah** | 484 | 224 | 32·1 | 244 | 0·54 | 0·47 | ,, | ,, ,, | Q | 4 |
| **Various crops** | **Quebec** | 174 | 175 | — | — | 0·34 | 0·20 | Summerby | *Tech. Bull. MacDonald agric. Coll.* (1934), **15** | — | 41 |
| Theoretical curve if correlation of adjacent areas were zero, or reduction of error obtainable with random replication ($b = 1·0$). | | | | | | | | | | T | — |

$b'$ is the slope of each regression estimated graphically from Fig. 4.

$b$ is the value estimated as appropriate for an infinitely large field as described in the text. As defined in this paper the coefficients refer to regression of *variance* on plot size. Published data, which were plotted on log-log paper for the preparation of Fig. 4, give invariably standard deviations or coefficients of variability. The slopes of regressions as shown in Figs. 4 and 5 are thus one-half of the values in the table.

C shows data for 2 years combined, 1909—O, 1910—X.

U, $b' = 0·80$ for plots 5 ft. wide, marked $\rho$, 0·58 for all other shaped plots, single row plots marked ○, intermediate shapes marked □.

WA and WB, error variance estimated for within blocks of 5 plots.

EA to ED, different strains of the same potato variety on adjacent areas of soil. Mean $b' = 0·42$, $b = 0·34$.

X, single row plots only within blocks of 6 plots.

Y, single row plots only within blocks of 5 plots.

FA to FD, multiple row plots. Single row plots shown as broken lines FE to FH (see text).

HA, within blocks of 12 plots.

HB, single row plots only within blocks of 12 plots.

L, plots 5 links wide marked $\rho$, $b' = 0·63$; other shapes O, $b' = 0·37$.

M, guarded plots, means of 6 years.

N to Q, unit plots = area occupied by a single tree. N to P in California, exact location given for reference in the publication concerned.

Irr. = irrigated. For. = forage.

as compared to a value-characteristic of the soil alone. It would be of theoretical interest to obtain coefficients characteristic of the soils alone, but it seems at present impossible to disentangle the effects of these incidental variables. This is, however, of little practical consequence, since for purposes of experimental planning they should be included. They will presumably vary with different crops.

The regression

$$\log V_x = \log V_1 - b' \log x,$$

when given the appropriate constants, may be considered to describe the soil and plant heterogeneity of an observed field. In order to compare the variability of plot yields for different crops and soils the regressions observed in Fig. 4 have been converted to show the regressions of the coefficient of variability on plot size in square feet. The results are shown in Fig. 5. The corresponding coefficients of variability for a standard size of plot (1/40th acre) and mean yields are given in Table II. The crops fall roughly into three groups: wheat, mangolds, beets, soybeans and sorghum (forage) seem to be least variable; maize, potatoes, sweet potatoes, cotton and natural pasture are intermediate; and fruit trees are most variable.

## 4. Variance within blocks

In order to bring results deduced from blank experiments into consonance with modern experimental practice we require to consider the variability of different sized plots within blocks. At first sight this complicates the problem, since the possible combinations of varying sizes and shapes of plots with varying sizes and shapes of block are very great. But if shape may be ignored and we have a general law giving the variability of plots of varying size, then an expression for variance between, and thence for that within, blocks can also be derived.

In §§ 2 and 3 we have considered simply variance of plots over the whole area of the fields used for the experiments. But size of field is purely arbitrary, and any given field may be considered as but a single block of a larger field. It is advisable, therefore, that the terms should be more accurately defined to indicate the area over which a variance is measured. Let the variance of the mean yield per unit area, of plots of $x$ units of area, over a block of $m$ plots be $(V_x)_m$. Then the regression which has been empirically observed for a field having $n$ plots is

$$\log (V_x)_{n/x} = \log (V_1)_n - b' \log x.$$

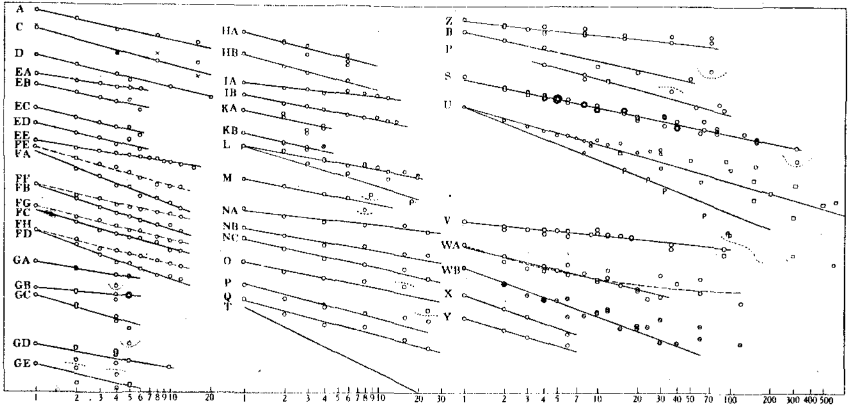


*Fig. 4.* ***Logarithmic relationship** between plot size and standard deviation per plot from data from blank experiments **reported in the literature.*** Ordinate: logarithm of standard deviation per plot ($\log s_x$). Abscissa: logarithm of size of plot ($\log x$). For key see Table II.

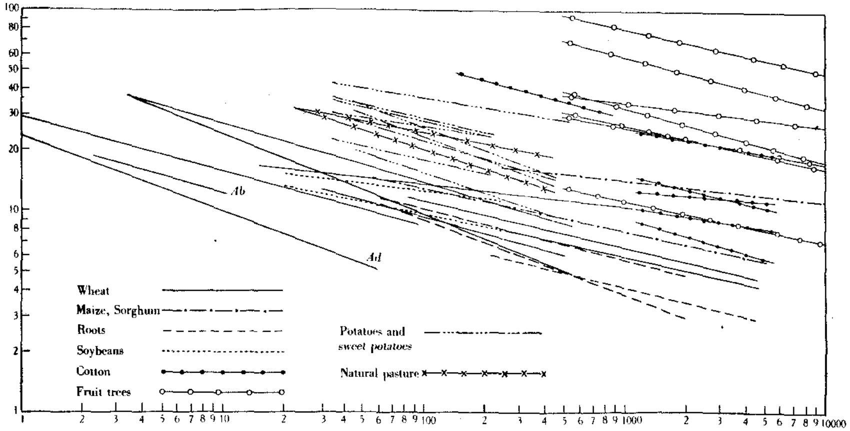


Fig. 5. Regressions of coefficient of variability on plot size in sq. ft. (Data in Table II.)
(43,560 sq. ft. = 1 acre.)

But the size of the field, $n$, is arbitrarily fixed, and if this regression is theoretically sound it should hold good for any alternative size of field which we may wish to consider as the area over which variances should be measured. But if $n$ be varied the above regression becomes curved for any value other than that originally assigned. The regression is therefore inconsistent with the requirement that the law shall be unaffected by variation in the size of the field.

This difficulty is overcome if we postulate an infinitely large field of which an observed field may represent a single block and suppose that the law be

$$\log (V_x)_\infty = \log (V_1)_\infty - b \log x, \qquad \ldots\ldots(1)$$

or

$$(V_x)_\infty = \frac{(V_1)_\infty}{x^b}. \qquad \ldots\ldots(1a)$$

When $b=1$ this gives the ordinary formula for the variance of the mean of $x$ independent units.

Since a block is merely a large-size plot the variance between blocks can be estimated from the same equation, and thence the variance of plots of $x$ units within blocks of $m$ plots is given by an analysis of variance, where $\lambda$ tends to $\infty$, as follows:

| | D.F. | Mean square |
|---|---|---|
| Between blocks | $(\lambda - 1)$ | $m\,(V_{xm})_\lambda$ |
| Within blocks | $\lambda\,(m-1)$ | $(V_x)_m$ |
| Total | $(\lambda m - 1)$ | $(V_x)_{\lambda m}$ |

thus in the limit

$$(V_x)_m = \frac{m\,(V_x)_\infty - m\,(V_{xm})_\infty}{m-1} = \frac{m\,(1-m^{-b})\,(V_x)_\infty}{m-1} \qquad \ldots\ldots(2).$$

For given values of $m$ and $b$, $(V_x)_m/(V_x)_\infty$ is a constant irrespective of $x$. Thus the association between neighbouring plots is of such a type that the variance per plot within a block of $m$ plots bears the same ratio to the total variance per plot in an infinite field whatever the plot size; or, alternatively stated, the intraclass correlation between groups of $m$ plots is the same whatever the size of plot.

On this hypothesis the variances recorded in §§ 2 and 3 should conform to a regression of the form

$$\log (V_x)_{n/x} = \log (V_1)_\infty - b \log x + \log \frac{n\,(n^b - x^b)}{n^b\,(n-x)}. \qquad \ldots\ldots(3)$$

This is a curve slightly concave downwards, but, as the curvature becomes appreciable only when $x/n$ approaches one-tenth and in this region the variances are not well determined, the curvature could not be detected by the methods used in the preceding sections.

The value of $b'$, however, estimated from a regression for a finite field will differ substantially from the equivalent $b$ for the infinite field.[1] The most expeditious way to convert the observed $b'$'s into equivalent $b$'s is to determine the corrections for a series of values of $b$ over the given range of $x/n$. This was done graphically. The magnitude of the corrections obtained is illustrated by the following table:

| $b$ | 1·0 | 0·8 | 0·7 | 0·6 | 0·5 | 0·4 |
|---|---|---|---|---|---|---|
| $b'$ in range $x/n$ from 0·001 to 0·01 | 1·0 | 0·804 | 0·710 | 0·617 | 0·528 | 0·443 |
| $b'$ in range $x/n$ from 0·01 to 0·1 | 1·0 | 0·822 | 0·738 | 0·656 | 0·578 | 0·504 |
| $b$ | 0·35 | 0·3 | 0·25 | 0·2 | 0·15 | 0·1 |
| $b'$ in range $x/n$ from 0·001 to 0·01 | 0·403 | 0·364 | 0·326 | 0·291 | 0·257 | 0·226 |
| $b'$ in range $x/n$ from 0·01 to 0·1 | 0·469 | 0·434 | 0·402 | 0·371 | 0·343 | 0·312 |

Equation (2) indicates that the expectation for the efficiency of a randomized block experiment having $m$ plots per block relative to one with $n$ plots per block is

$$\frac{(V_x)_n}{(V_x)_m} = \frac{n\,(m-1)\,(1-n^{-b})}{m\,(n-1)\,(1-m^{-b})}. \qquad \ldots\ldots(4)$$

As a guide to the effect on experimental error of changing the size of block, the curves for $(V_x)_m$, taking $(V_x)_\infty$ equal to unity, for various values of $m$ and of $b$ are plotted in Fig. 6. As an example of the use of these, suppose we wish to conduct an experiment with forty treatments and we want to know if it is worth while to adopt a complex design by which block size may be reduced (for example, by confounding interactions or "pseudo-factors" (Yates, 1935, 1936)) as compared to a simple lay-out using blocks of forty plots. If we expect the field to show a "$b$ coefficient of heterogeneity" of 0·2 the expectation for relative efficiencies of 5, 10, 20 and 40 plot blocks is

$$\frac{0{\cdot}535}{0{\cdot}344} : \frac{0{\cdot}535}{0{\cdot}410} : \frac{0{\cdot}535}{0{\cdot}475} : \frac{0{\cdot}535}{0{\cdot}535} = 1{\cdot}56 : 1{\cdot}30 : 1{\cdot}13 : 1,$$

and it will be worth considerable effort to be able to use small blocks. But if the $b$ coefficients be about 0·7 the expectation of relative efficiencies is only

$$1{\cdot}12 : 1{\cdot}06 : 1{\cdot}03 : 1,$$

and it would be best to retain the simplicity attaching to the larger blocks with a simple design.

In a similar manner to the derivation of (2) above it can be shown

[1] It should be noted that an observed value of $b'$ depends in part on the number of unit plots observed. This condition can be demonstrated from actual data, or may be deduced from an hypothesis that a linear logarithmic regression may be applicable for a finite field of given size, as well as from the law for a theoretical infinite field.

that where differential directional heterogeneity is not present (so that shape of block is unimportant) the error variance in an $m \times m$ latin square may be represented by

$$(V_x)_{m_2} = \left\{\frac{m(1-m^{-b})}{m-1}\right\}^2 (V_x)_\infty .$$

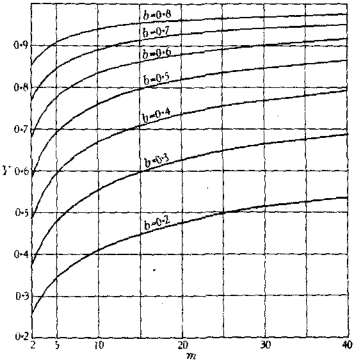


Fig. 6. To show reduction of error variance obtainable by subdividing an area into blocks for different values of the $b$ coefficient of heterogeneity and of the number of plots per block ($m$). The ordinate, $Y = (1-m^{-b})/(1-m^{-1})$, shows the ratio of error variance within blocks to variance over an infinite area.

The frequency distribution of the adjusted $b$ coefficients given in Table II is

| Adjusted $b$ | 0·05–0·15 | 0·15–0·25 | 0·25–0·35 | 0·35–0·45 | 0·45–0·55 | 0·55–0·65 | 0·65–0·75 | 0·75–0·85 | Total |
|---|---|---|---|---|---|---|---|---|---|
| Frequency | 4 | 4 | 8 | 6 | 7 | 4 | 3 | 1 | 37 |

The mean is about 0·4, but since the limiting values of $b$ are 0 and 1 and three-quarters of this range is covered by the observations, the diversity of conditions is very great.

## 5. The optimum size of plot

From equations (1a) and (2) the information per plot of size $x$ in blocks of $m$ plots is

$$\frac{1}{(V_x)_m} = \frac{(m-1)}{m(1-m^{-b})} \frac{x^b}{(V_1)_\infty}.$$

The cost per plot may be given by a linear regression

$$K_1+K_2x,$$

whence the cost per unit of information is

$$\frac{m\,(1-m^{-b})\,(K_1+K_2x)\,(V_1)_\infty}{(m-1)\,x^b}. \qquad \ldots\ldots(4)$$

This is minimum when

$$x=\frac{bK_1}{(1-b)\,K_2}. \qquad \ldots\ldots(5)$$

This provides a formula for estimating the most efficient size of plot for any given experiment. It is not affected by the number of plots, which may be determined either by the area available or by the number which will probably be necessary to reduce the error variance of treatments to any assigned figure.

## 6. Guarded plots

In experimental work it is customary to discard guard areas around all plots. The effect of this condition has been ignored in the preceding discussion.

Suppose that an area be divided into $n$ plots side by side, of which each alternate plot may be taken as the guard area. Then if $n$ is even we have the following analysis of variance:

| | D.F. |
|---|---|
| Between experimental plots, $E$ | $\frac{1}{2}n-1$ |
| Between guard plots, $G$ | $\frac{1}{2}n-1$ |
| $\bar{E}-\bar{G}$ | 1 |
| Total | $n-1$ |

The mean square for $\bar{E}-\bar{G}$ is likely to be smaller than the other two components, since it depends on the comparison of neighbouring plots, but if $n$ be large the mean squares between $E$ and between $G$ will be only slightly larger than the mean square for the total, and may for practical purposes be regarded as approximately equal to it.

Extending this argument, we may say that if $m$ guarded plots each with "test-area" $x$ occupy the same total area as $m'$ unguarded plots of the same area $x$, then the variance within blocks of the $m$ guarded plots will be equal to $(V_x)_{m'}$. The procedure of § 5 may thus be simply modified so as to be applicable to guarded plots.

Thus, if a block consists of a single row of plots side by side, the end guards (area A) may be considered as lying outside the block. The area occupied by the side guards bears to the "test-area" $x$ the ratio $B=(W-w)/w$ where $w$ is the width of the test area and $W$ the width

of the test area plus guards. If $K_g$ is the cost per unit area for handling guard areas, the cost per plot may be given by:

$$K_1 + K_2x + K_g(A + Bx).$$

Thus, proceeding as before, the cost per unit of information is found to be a minimum when

$$x = \frac{b(K_1 + K_gA)}{(1-b)(K_2 + K_gB)}. \qquad \ldots\ldots(6)$$

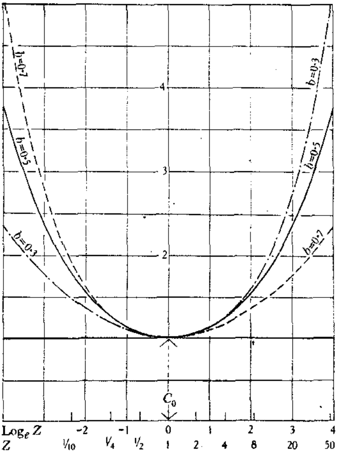


Fig. 7. To show increase of cost (or of error variance) if the optimum size of plot be not used. Ordinate: cost relative to cost using the optimum size of plot. Abscissa: logarithm of size of plot relative to optimum size.

## 7. Cost of using plot sizes other than the most efficient

Equation (4) gives the cost per unit of information as

$$\text{Const.}\,(K_1 + K_2x)\,x^{-b},$$

which is minimum when

$$x = \frac{bK_1}{(1-b)K_2} = x_0.$$

If the most efficient size plot be not used the relative cost is

$$y = \frac{\text{cost}}{\text{min. cost}} = bz^{(1-b)} + (1-b)\,z^{-b} \qquad \text{......(7)}$$

$$= be^{(1-b)\ln z} + (1-b)\,e^{-b\ln z}, \qquad \text{......(8)}$$

where $z$ is $x/x_0$, that is the ratio of the size of plot used to that size which is most efficient. The second form (8) is the more convenient both for calculation and to give a symmetrical scale for relative plot sizes. It is of interest to note that if $b=0{\cdot}5$, (8) is the equation of a catenary curve. Fig. 7 gives the curves for $b=0{\cdot}3$, 0·5 and 0·7. When $b=0{\cdot}5$ efficiency is

96 % if plots are double or half the optimum size.
80 % if plots are quadruple or quarter optimum size.
63 % if plots are eight times or an eighth optimum size.
47 % if plots are sixteen times or a sixteenth optimum size.

The value of $b$ does not greatly affect these estimates in the region a quarter to four times the optimum size. Beyond this range if $b$ is less than 0·5 it is more serious to have plots which are too large than to have plots which are too small, and vice versa.

## 8. Arithmetical example of estimating the most efficient plot size for a given experiment

In analytical yield trials it is required to count plant numbers, tiller numbers and ear numbers, weigh straw and grain, and estimate weight per grain. The $b$ coefficient of the Canberra experiment field has been estimated to be about 0·75. Sowing with a Woodfield dibber fixes the rows (width of plot) at 4 ft., and rows are 6 in. apart. Six inches around each plot are discarded as guard. We thus have $A=4$ sq. ft., $B=0{\cdot}33$. Costs other than labour are relatively negligible, and it is assumed that the same class of labour is used throughout. From past experience it has been estimated that, on the average,

| | | | | | |
|---|---|---|---|---|---|
| (1) | Preparing seed | requires | 0·005 | man hour | per sq. ft. |
| (2) | Sowing | ,, | 0·017 | ,, | sq. ft. |
| (3) | Counting plants at braird | ,, | 0·002 | ,, | sq. ft. |
| (4) | Counting tillers | ,, | 0·062 | ,, | sq. ft. |
| (5) | Observing earing and flowering dates | ,, | 0·033 | ,, | plot |
| (6) | Counting plants near harvest | ,, | 0·01 | ,, | sq. ft. |
| (7) | Cutting out guards | ,, | 0·001 | ,, | sq. ft. |
| (8) | Harvesting and weighing sheaf | ,, | 0·030 | ,, | sq. ft. |
| (8a) | | | 0·025 | ,, | plot |
| (9) | Counting ears | ,, | 0·033 | ,, | sq. ft. |
| (10) | Threshing | ,, | 0·016 | ,, | sq. ft. |
| (10a) | | | 0·016 | ,, | plot |
| (11) | Weighing grain | ,, | 0·016 | ,, | plot |
| (12) | Estimating average weight per grain | ,, | 0·06 | ,, | plot |
| (13) | Statistical analysis | ,, | 0·10 | ,, | plot |

Whence

$K_1 = (5) + (8a) + (10a) + (11) + (12) + (13) = 0{\cdot}25$ man hour per plot.

$K_2 = (1) + (2) + (3) + (4) + (6) + (8) + (9) + (10) = 0{\cdot}18$ man hour per sq. ft.

$K_g = (1) + (2) + (7) = 0{\cdot}023$ man hour per sq. ft.

$$x = \frac{b\,(K_1 + K_g A)}{(1-b)(K_2 + K_g B)} = \frac{0{\cdot}75\,(0{\cdot}25 + 0{\cdot}023 \times 4)}{0{\cdot}25\,(0{\cdot}18 + 0{\cdot}023 \times 0{\cdot}33)} = 5 \text{ sq. ft.}$$

## 9. DISCUSSION

The regression of plot variance on size is an empirical relationship for fields of a size normally considered in experimental work. Since it implies that adjacent areas are equally correlated irrespective of their size and this condition must sooner or later break down, the relationship cannot be extended indefinitely. It nevertheless appears to provide a method of evaluating approximately the average relative efficiencies of varying sizes of plots and of blocks. The wide range of values of $b$ coefficients is a measure of the variation in types of soil heterogeneity. A similar degree of variation is demonstrated by the estimates of efficiencies of various randomized block and latin square experiments reported by Yates (1935). It is consequently impossible to forecast accurately the relative efficiencies of different arrangemeuts for a field of whose heterogeneity little or nothing is known. One might perhaps anticipate that the $b$ coefficients should be low for fields known to be heterogeneous and high for fields of fairly even fertility, but the absence of correlation between $b$ and coefficients of variability in Table III indicates that any such assumption may not in fact be justified. So far as present evidence goes total variability and the manner in which varying fertilities are distributed appear to be two distinct features which must be separately considered in any quantitative measure of soil heterogeneity. It is to be noted, however, that the optimum plot size and the increase in information resulting from reduction in the number of plots per block is dependent only on the value of $b$.

Information about the persistence of similar values of $b$ for a given field over several years and with different crops is required before we can say how far it may be worth while to determine coefficients appropriate for fields which are to be frequently used for experimental work. No evidence on this point is at present available,[1] except that if heterogeneity

[1] Data which might be used to test this question have been given by Summerby (1934) for oats, alfalfa and maize in 3–5 years, and by Garber *et al.* (*J. agric. Res.* (1926), **33**, 255–68) for oat hay and wheat grain in 2 years, and (*J. Amer. Soc. Agron.* (1931), **23**, 286–98) for maize, oats and wheat in 3 years. The present writer is, however, unable to devote to this problem the time required for the necessary calculations.

types were persistent one might perhaps expect the covariance of plots in successive years to be more useful than it has been found to be.

Since the regression of variance on plot size is a function of the correlation of adjacent areas, it appears theoretically inevitable that shape of plot should have some effect, since the correlation of ends of long narrow strips must usually be less than that at opposite sides of a square of equal area. The shapes of plots considered may therefore be expected to have some effect on the regression. The importance of plot shape is of course particularly accentuated in fields which show differential directional heterogeneity (e.g. U and L). In a field where the orientation of the variability is known and is reasonably persistent, long narrow plots with a particular orientation will obviously be called for. In such circumstances it might be worth while to make use of two $b$ coefficients with assigned directions to describe the heterogeneity; one being applicable for estimates regarding size of plot, the other for considerations of block size. The effect of different shapes of plots or of blocks appears to be responsible for the fact that Yates (1936) using data from Parker & Batchelor obtained greater efficiency for small blocks than would be indicated by the regression $M$ of Fig. 4 which is based on only four variances given by the original authors.

## 10. Summary

Using data from a blank experiment with wheat it was found that the regression of the logarithms of the variances for plots of different areas on the logarithms of their areas was approximately linear. A graphical review of variances, etc., reported in the literature for thirty-nine other blank experiments indicates that the results of most such experiments conform to the same law.

It is shown that the above law can be generalized (so as to be applicable to any size of field) by applying a certain adjustment to the regression coefficient $b'$, so as to give a modified coefficient $b$ applicable to an "infinite" field.

From this generalized relationship there has been deduced an expression ((4), p. 16) to indicate average relative efficiencies to be expected for randomized block experiments with varying numbers of plots per block in a field for which the coefficient $b$ is known.

A formulae (5), which may be used to estimate the most efficient size of plot for any given experiment, has also been deduced. The cost of using plots of other than the most efficient size is indicated graphically in Fig. 7.

## 11. ACKNOWLEDGEMENTS

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# THE FUNCTION OF THE CUTICLE IN RELATION TO THE POROSITY OF EGGS

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(With Plate I and Three Text-figures)

## INTRODUCTION

THE porosity of egg-shell, which may be defined as that property of the shell which permits of the exchange of water vapours and gases between the contents of the egg and the outer atmosphere, arises from the presence in the shell of numerous pores. These latter are well organized structures and traverse the entire thickness of the shell, opening on the exterior at the centre of slightly depressed or countersunk areas. Numbering about 6000–8000 per egg, they contain protein material and have an average diameter of 0·038–0·054 mm. Over their countersunk exits the cuticle, which is elsewhere quite entire, shows small, radiating cracks or fissures and if some dye is placed in the interior of a broken egg, it penetrates the pores and stains the cuticle immediately around the exits, demonstrating the continuity of the protein content of the pore and the cuticular structure. The effective evaporating surface of an egg is therefore not the total area of the pore exits but the total area of the stained cuticular areas surrounding these exits, two areas which stand to each other in the ratio of about 1 : 13. Thus, contrary to popular belief, the removal of the cuticle should *retard* evaporation and we will present evidence to show that observed porosity values can be explained quantitatively only when allowance is made for the considerable influence of the cuticle in increasing evaporation.

## LITERATURE

There are several ways in which porosity may he studied: broadly these are: (1) Governing factors, (2) Immediate results, (3) Functional

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importance, (4) Measurement. These may be expanded in tabular form:

- Physical factors governing porosity
  - Cuticle
  - Shell
    - Thickness
    - Structure and composition
    - Pores
  - Lining membranes
  - Outer atmosphere
    - Ventilation
    - Temperature
    - Humidity
- Immediate results
  - Loss in weight
  - Development and growth of air spaces
- Functional importance
  - Growth and development of embryo
  - Storage of eggs
  - Control and variation of incubating conditions
- Measurement by
  - Loss in weight
  - Development of air space
  - Shell texture (candling)
  - Staining with dyes
  - Penetration of air and gases

### (1) *Physical factors governing porosity*

*Cuticle.* The cuticle or "bloom" is the film which covers the exterior of the egg. With regard both to its nature and function there appears to be divergent opinion. It has been described as "somewhat porous but apparently quite structureless otherwise...at the same time the cuticle seals up the pores of the shell to a degree, thereby retarding evaporation. It is quite soluble and is destroyed when an egg is washed" (Lippincott & Card, 1934). This epitomizes the view generally held. On the other hand it has been described as having a much more organized structure, with a deep layer containing nucleated cells and having what appears to be a basement membrane, removable only by acids. The deep layer covers the pore entrances, though in this region they show a marked cracking or drying, thus enormously increasing the porosity of the shell (Stewart, 1936). Regarding solubility, it has been shown that eggs washed by a variety of solutions do not show any increased porosity, i.e. increased loss in weight, and that where the cuticle is removed by acid the porosity is actually decreased (Bryant & Sharp, 1934). In fact these authors conclude "...that washing eggs does not remove the protecting film from the surface of the shell and that if it did the eggs would not lose moisture faster than if it were present".

*Shell structure and composition.* The shell, which consists chiefly of calcium carbonate crystals in the form of calcite and of calcium phosphate, shows three distinct layers. In the outermost, the calcite crystals are compactly arranged with their long axes at right angles to the surface.

The intermediate, or spongy layer, an aggregate of randomly orientated calcite crystals, constitutes two-thirds of the entire thickness and is described as being porous and admitting the passage of air and water. The innermost, or mammillary layer, is constructed of spherules of calcite more or less separated from each other and the spongy layer by a series of interstices, communicating on the one hand with the fibres of the shell membrane and on the other with the pores of the spongy layer (Stewart, 1936; Lillie, 1919).

Careful analysis of egg-shells has failed to demonstrate any chemical differences between porous and non-porous shell (Dunn, 1923*b*).

*Pores.* These are definite, well-developed structures which traverse the entire thickness of the shell; they must not be confused with the irregular "mesh" of the spongy layer, from which they are quite distinct. They commence at the mammillary layer and open on the outer surface at slightly countersunk areas into which the cuticle dips, though some stop short of the surface, their course being abruptly aborted. They vary in diameter from 0·038 to 0·054 mm. (Stewart, 1936), although some authors describe two types of pore, small and large (Bryant & Sharp, 1934). They number from 6000 to 8000 per egg and are fairly uniformly distributed over the shell (Almquist & Holst, 1931).

The use of chemical tests shows that they contain protein material (Stewart, 1936), and the application of dyes that this protein is continuous on the one hand with the deeper layers of the cuticle, and on the other with the lining membrane of the shell. Their presence can be easily demonstrated by a number of methods which will be considered below.

Their mode of formation does not appear to have been fully studied but it has been suggested that they arise from the inclusion in the crystallizing mass of calcite of numerous threads of protein material. In view of the importance of porosity for the natural development of the chick, it seems likely that a less haphazard origin is indicated, the regularity of distribution alone making it unlikely that they arise by fortuitous coincidence. Again, all the pores run at right angles to the surface and not with an irregular inclination. It seems feasible that some positive agency must contribute to the pre-arrangement of the protein fibres around which the pores develop, that strands may pass between the deep cellular layer of the cuticle and the outer lining membrane of the shell, *before* calcification occurs. This point awaits elucidation.

The number of the pores is closely related to the porosity, the greater the number the greater being the porosity; the relationship between the two is linear (Almquist & Holst, 1931).

*Thickness.* This factor must be considered, though it is doubtful whether it has much significance. It may operate in two ways. First, if pores are formed by the haphazard inclusion of fibres (*vide supra*) then there is a greater probability that thin-shelled eggs will have a larger number of pores. This is upheld, indirectly, by the experiments of Dunn (1923*a*), who shows that large eggs are, on the average, less porous than small eggs, probably due in part to the thicker shell. Secondly, thickness is of interest as an explanation of the variation of pore numbers, e.g. it was noted above that the number of pores increases as incubation proceeds (Almquist & Holst, 1931), and this almost indubitably arises from the thinning of the inside of the shell by lime absorption for foetal growth. Again, if the cuticle is removed by sand blasting (Bryant & Sharp, 1934), a method sometimes used for the dry cleaning of eggs, the number of pores may be increased by as much as 200 per cent, presumably due to the thinning of the outside of the shell and the exposure of the outer ends of aborted pores. It is of particular moment to the present research to observe that this considerable increase in pores was accompanied by only a 20 per cent increase in porosity, an apparent inconsistency which is easily resolved on the concept of our conclusion, viz. that the cuticle itself plays the major role in determining porosity, and that the pores are only incidental to that end.

*Membranes.* The two lining membranes of the shell do not appear to have been studied in their relation to porosity. The outer, which adheres firmly to the mammillary layer and whose fibres, if one can judge by the action of dyes, are in direct communication with the protein of the pores and cuticle. It is 0·05 mm. thick and much coarser in texture than the inner membrane which is only 0·015 mm. thick (Hays & Sumbardo, 1927). Any moisture which escapes from the egg must in the first place pass through both membranes by capillary attraction before reaching the pores. At the rounded end of the egg, the two membranes, which are elsewhere in contact, separate to form the air space. It has been suggested that while shell porosity is not necessarily greater over the air space, it is to be expected that this will be so in older eggs, as there is more chance of drying in this region (Almquist & Holst, 1931).

*Atmospheric conditions, etc.* Humidity can be measured as "absolute", which expresses the tension of water vapour present in a given atmosphere, or as "relative", which expresses the water vapour present as a percentage of that required to saturate the atmosphere at the particular temperature involved. Evaporation from eggs, i.e. porosity, is directly proportional to the latter quantity.

Temperature variations affect the losses to a lesser extent, the porosity figures increasing slowly with rise of temperature (Pringle & Barott, 1937).

The time of holding and the treatment of individual eggs also influence porosity (Almquest & Holst, 1931). All these various factors have been kept constant in our experiments.

### (2) *Immediate results*

As the water evaporates the egg loses weight and the resulting shrinkage of its contents leads to the progressive growth of the air space. At the end of the incubation period the egg may have lost 10 per cent or more of its weight. Most workers find that under set conditions the rate of moisture loss from both fertile and unfertile eggs remains constant throughout the greater part of the incubation period (Pringle & Barott, 1937; Bryant & Sharp, 1934), but Dunn (1922–3*a*) reports a slowing off of the rate during the latter half of incubation.

### (3) *Functional importance*

The existence of shell porosity is of vital importance, for, quite apart from the water losses which to a considerable extent must influence the delicate changes of chemical balance in the developing tissues of the embryo chick, the interchange of respiratory gases via the blood vessels of the allantois which ultimately lines the shell could not occur if the shell were impervious.

It must also be obvious that there are limits to a functional porosity range; an upper beyond which the porosity becomes so great that water loss is too rapid for natural embryonic growth, and a lower below which a sufficiency of respiratory interchange is impossible, and where water loss is too small to permit the development of an adequate air space. Speaking of these limits, Dunn (1923*c*) says: "There are limits beyond which porosity and evaporation lead to death, but these limits are far apart and are seldom approached in eggs which are not visibly abnormal... and we cannot conclude that the variations in the rate at which eggs lose weight is a major factor in embryonic mortality."

The storage quality of egg is also dependent upon the porosity. "Porosity or lack of porosity must be assumed to be one of the important factors bearing on commercial egg quality and particularly on the preservation of eggs, excessive porosity leading to excessive drying or added risk of infection of the contents" (Almquist & Holst, 1931).

(4) *Measurement*

Porosity may be measured in a variety of ways. The most useful for those concerned with incubation practice is the study of the air space as revealed by candling, and the most accurate for scientific purposes is the estimation of the rate of loss in weight under standard conditions of humidity and temperature (Bryant & Sharp, 1934; Pringle & Barott, 1937). For pore estimation the entire egg may be immersed for 3 min. in an alcoholic solution of methylene blue, removed, dried and cracked open when it will be found that the lining membrane shows punctate staining, the number of pores being more or less clearly shown (Almquist & Holst, 1931). The chief drawbacks to this method are that (1) it does not imitate the actual process or conditions of porosity, (2) its scale of values is quite arbitrary and must be transposed into weight loss values, (3) the egg has to be destroyed, (4) examination of the stained membrane shows large numbers of definite spots but also large numbers of very faint spots occasioned by staining only of the outer-lining shell membrane. How to interpret this mixed presentation as definite porosity values is problematical. The advantage of the method is the easy ocular demonstration of pores.

A method for the study of porosity towards gases is to saturate the addled egg-shell membranes with lead acetate, seal the opening and then expose the egg to sulphuretted hydrogen. The gas penetrates the pores and gives rise to dark spots of lead sulphide in the membrane over the inner ends of the pores (Stewart, 1936).

Again, if an egg is placed in some liquid and the whole subjected to a strong negative pressure streams of air bubbles issue from the pores; but the number of pores revealed by this method are relatively small, e.g. 18 compared with the 8000 found by using dye (Bryant & Sharp, 1934). This marked discrepancy may be explained, we think, on two grounds: (1) where the pores vary in size the capillary forces of the finer pores are considerably higher than that of the larger pores; hence gases will escape by preference via the latter; (2) the negative pressure may draw the lining membrane against the inner opening of the pores and so effectively seal them off.

The reverse phenomenon, i.e. gas issuing under positive pressure, occurs when an egg is placed in boiling water, the expanded air then issuing in a stream of bubbles from the larger pores.

## Experimental determination of porosity

(1) Porosity has been defined as that property of shell which permits of the interchange of gases and water vapour between the contents of the egg and the outer atmosphere.

(2) The measurements undertaken aim at determining the rate of loss of water per unit area of shell, in eggs kept under standardized conditions for definite periods; to this end it becomes necessary to calculate the surface area of an egg.

A formula for this purpose has already been suggested by Dunn (1922–3*b*) but as his formula was primarily designed for use with statistical data, we have thought it necessary to derive a more accurate method for the present group of experiments.

(3) Generally speaking, an egg can be described as a prolate spheroid whose minor axis cuts the major axis, not centrally, but towards the round end of the egg. Mathematically, an egg of uniform curvature can be regarded as consisting of two hemi-prolate spheroids, differing in their eccentricities and meeting in the plane of their common minor axes.

(4) This concept deduced on general grounds has been checked in two ways:

(*a*) Using a special instrument, accurate tracings were taken of five varied eggs, representing plane sections along their length. The two axes were then inscribed, the minor being drawn across the widest part of the tracing. The theoretical ellipses having the axis $a_p$, $b$ and $a_t$, $b$, were superimposed by geometrical construction. In three cases the congruence of the curves was perfect and in two there was a small divergence from the theoretical form in the pointed half of the egg, in degree amounting to about 1 per cent of the major axis length.

(*b*) The long perimeter of another five eggs was measured by placing a spot of ink on the point and rolling on blotting-paper, the average distance between two successive dots giving the perimeter. The theoretical perimeter for the two hemic ellipses $a_p b$ and $a_t b$ was then calculated from the formula

$$\pi (a+b) \left[1+\frac{R^2}{4}\right] \quad \text{where} \quad R=\frac{a-b}{a+b}.$$

The agreement between the values was remarkably good.

(5) The length and breadth measurements are made with callipers, though it is simpler to place the egg between two accurately cut wooden blocks and measure their distance apart. $a_t$ is found by resting the

vertically placed egg on a horizontal plane and measuring the tangential distance.

Table I. *Agreement between the calculated and observed long perimeter of eggs*

| Egg | Perimeter observed | S.D. | Calc. | Error (%) |
|---|---|---|---|---|
| 1 | 15·18 | ±0·016 | 15·29 | 0·725 |
| 3 | 19·00 | 0 | 19·1 | 0·527 |
| 5 | 15·025 | ±0·025 | 15·15 | 0·825 |
| 7 | 14·49 | ±0·015 | 14·64 | 1·0 |
| 9 | 14·45 | 0 | 14·48 | 0·014 |

(6) To determine and record the shape completely three measurements are required, viz. the length, the maximum breadth and the point at which these two axial measurements intersect. These values are then described as

Length $=2a$

Breadth $=2b$

Top of egg to maximum breadth $a_t$

Point of egg to maximum breadth $a_p$

Obviously $a_p=2a-a_t$.

(7) The surface area of a prolate spheroid is given by the formula

$$A_c=2\pi b^2+2\pi\frac{ab}{e}\sin^{-1}e, \qquad \ldots\ldots(1)$$

where
$$e=\frac{\sqrt{(a^2-b^2)}}{a}.$$

To find the area of the egg one must therefore find the respective surface areas of the two hemi-prolate spheroids $a_t$, $b$ and $a_pb$ and add these together.

It has been found by experiment that the same result follows (within 0·2 per cent) by calculating the surface area of the single hypothetical prolate spheroid $2a/2$, $b$. This method of calculation has therefore been adopted as it obviates the need for the troublesome measurement $a_t$.

(8) The observed and calculated volumes of thirty eggs have been compared. The average error was 3·2 per cent, the maximum 12 per cent and the minimum 0·33 per cent. The calculated values were always larger than the observed.

(9) A correction for errors of measurement and small departures from the theoretical form is next applied. The volume of the egg calculated from

$$V_c=\tfrac{4}{3}\pi ab^2 \qquad \ldots\ldots(2)$$

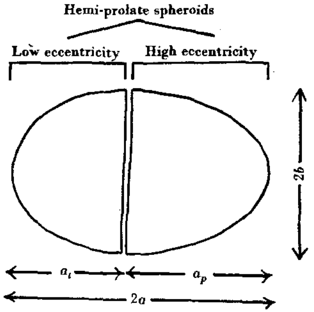


Fig. 1. Ideal mathematical form of egg.

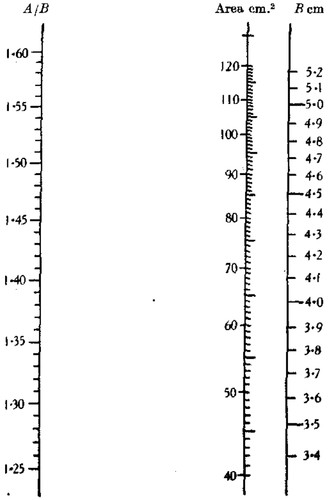


*Fig.* 2. Nomogram for surface area of egg. By the use of this nomogram one may obtain the uncorrected surface area ($A_c$) with an error not exceeding 1 %. The method of finding from this the corrected area ($A_0$) is described in the text. $A$ = length of egg in cm.; $B$ = breadth of egg in cm.

is compared with the actual volume as determined by specific gravity methods ($V_0$). As the surface calculation also involves only the two constants $a$ and $b$ it is assumed that the value $A_c$ can be corrected as follows:

$$A_0 = A_c \left(\frac{V_0}{V_c}\right)^{\frac{2}{3}}, \qquad \ldots\ldots(3)$$

the surface being proportioned to the $\frac{2}{3}$ power of the volume. Provided the percentage difference between $V_c$ and $V_0$ does not exceed 4 per cent, then the error of the corrected surface area will not exceed 1 per cent.

(10) Summarizing the method of determining surface area. Measure the length ($2a$) and breadth ($2b$) of the egg. Calculate the surface area from formula (1). Next find the actual volume ($V_0$) by specific gravity method and calculate volume ($V_c$) from formula (2). Finally find the corrected area ($A_0$) by formula (3).

N.B. Choose only eggs of even curvature and discard eggs which give a $V_0 \sim V_c$ difference of more than 4–5 per cent.

(11) The standard conditions under which loss of weight is observed relate to temperature and humidity. The egg is placed in a small desiccator over anhydrous calcium chloride. (Relative and absolute humidity are therefore zero) and left in an incubator at 37° for approximately 24 hr. The porosity coefficient is then calculated as follows:

$$\text{Porosity coefficient} = \frac{\text{Loss of wt. in mg.}}{\text{Surface area in cm.}^2} \times \frac{24}{\text{Time in hours}}$$

## APPLICATION OF THE METHOD

Partly with a view to establishing standards for later measurements, but also to test the general value of the method by comparing our findings with those of existing literature, we examined several groups of eggs. In each instance the eggs were studied on the day of laying and apart from the porous group, which were deliberately selected because of their defects, all eggs were carefully examined by inspection and candling in order to rule out any unusual features such as body cracks, etc.; the groups therefore, each of which contained about sixteen eggs, represent carefully selected eggs: these were:

(1) Brown eggs of average incubation size. (Medium brown.)

(2) Brown eggs smaller than incubation size. (Small brown.)

(3) Brown eggs larger than incubation size. (Large brown.)

(4) White eggs of average incubation size. (Normal white.)

(5) White eggs selected on account of their defective structure. (Porous white.)

In addition the thickness of the shells of all the brown eggs was measured by the use of a screw micrometer gauge. In making this measurement a piece of shell was broken from the equatorial region of the eggs and the average of three measurements taken. Earlier, measurements were averaged from twenty-four readings on four pieces of shell from each egg, taken one from each pole and two from the equatorial region, but as no significant or systematic variations were observed in shell thickness from different regions of the eggs, the technique was simplified to that first mentioned. Both membranes were carefully stripped before measuring.

Table II. *Experimental porosity values*

| | Porosity | Porosity range | No. of eggs | Standard error | Thickness (inches) |
|---|---|---|---|---|---|
| (1) Brown medium | 4·8637 ±0·177 | 3·96 – 6·19 | 16 | ±0·0442 | 0·01363 |
| (2) Brown small | 5·68 ±0·737 | 4·77 – 7·37 | 17 | ±0·179 | 0·01275 |
| (3) Brown large | 6·01 ±1·225 | 4·70 – 8·36 | 15 | ±0·317 | 0·01332 |
| (4) White medium | 6·1125 ±0·87 | 5·02 – 7·34 | 16 | ±0·217 | — |
| (5) White *porous* | 7·3021 ±3·93 | 3·18 – 16·35 | 14 | ±1·05 | — |

Table III. *Experimental percentage loss values*

| | % loss | Range | S.E. |
|---|---|---|---|
| Brown medium | 0·5592 ±0·060 | 0·472–0·706 | ±0·015 |
| Brown small | 0·7126 ±0·095 | 0·570–0·921 | ±0·023 |
| *Brown large* | 0·6564 ±0·134 | 0·471–0·923 | ±0·035 |
| Brown medium and large | 0·6076 ±0·097 | — | ±0·0175 |

The values used in the following comparisons will be found in the accompanying table. The comparisons studied are:

(1) Brown with white.

(2) Porous with non-porous.

(3) Three sizes of brown eggs, with special bearing on the influence of shell area and influence of shell thickness on porosity.

(1) Comparing medium-sized brown and white eggs it will be noted that the former are less porous than the latter. This agrees with the observations of others (Gilbert, 1894; Laurie, 1911).

(2) The examination of defective or porous eggs showed that while eggs which to general tests appeared defective were not always more porous than eggs showing normal texture, there were in this group several eggs showing very high porosity coefficients. In general these latter were eggs which had very rough shells, where the normal process of calcification had obviously been markedly upset. We attempted to correlate the variations of light transmission through an egg at candling with the porosity.

Eggs candled before a strong light frequently display variations in the amount of light transmitted through different areas of the same shell. The areas responsible for this higher light transmission may be either in the form of isolated "flecks" or of flecks amalgamated into areas of considerable dimensions. In extreme cases the area of high light transmission may extend to the greater portion of the total shell area, imparting the appearance of high-grade fragility. Such eggs are popularly referred to as "thin-shelled" and are rejected by poultrymen when selecting eggs for hatching. The term "thin-shelled" is adequate since it at once describes the effect and notes the underlying cause, the mal-accretion of calcium. But if poultrymen agree in rejecting much-flecked and thin-shelled eggs, unanimity is wanting when underlying reasons are discussed.

There are two chief schools of thought, one holding that the mal-accretion and consequent danger of calcium deficiency is the real reason of rejection; the other side reject such eggs on the ground that a thin shell means a higher degree of porosity, and excessive porosity is undesirable. Experiments were conducted to ascertain whether any relation exists between porosity and high light transmission.

*Material.* Eggs were candled and selection made of those displaying marked light-transmitting areas. Both white and brown eggs were included although the latter displayed only few and isolated flecks. The whites ranged from isolated flecks up to areas covering the greater part of the total shell surface.

*Method.* Eggs were candled over a 100 W. lamp and flecks ringed with pencil. In the case of the brown eggs every fleck was ringed. With the whites, owing to the very large number of flecks and light-transmitting areas only the larger flecks and areas were ringed, but in no case where the numbers of flecks permitted, were fewer than 100 such areas ringed. The eggs were then carefully opened, by means of a fine-sawing edge, through the short axis and the halves washed out. Care was taken to preserve intact the membranes from one half, those from the other being removed. The shells were then set to dry for varying periods, after which each half was filled with a suitable stain and left until penetration of the stain marked the pore exists. The stain was then siphoned off and the shells set to dry. Penetration usually takes place within 3 min., warmth of the shell assisting the process, although one brown shell failed to show penetration after 7 min. If the stain be allowed to remain too long blurring of the pores results. If well timed, every pore shows up clearly as a pin-point of blue. It was thus a matter of calculation as to

whether the ringed areas showed a higher percentage of pores than the denser areas of the shell, as required by the assumption noted above. In no count could this be said to be borne out. No ringed area yielded a count higher than expectancy, and many rings remained unstained.

The inference is that there does not appear to be any ground for the supposition that areas of high light transmission carry a higher porosity factor than do areas with a lower degree of light transmission, if the number of pores be accepted as a criterion of porosity. Bryant & Sharp (1934) find a coefficient of correlation of $+0{\cdot}172 \pm 0{\cdot}098$ between the candling texture on the 20th day and the pores count.

(3) The comparison of various values for the three groups of brown eggs is more intricate and necessitates a brief explanation of some concepts involved. A small egg, in proportion to its weight or volume, has a larger snrface area than is the case for large eggs, i.e. the ratio $\frac{\text{surface area}}{\text{weight}}$ is greater for the small eggs. Naturally the actual surface area is greater the larger the egg. Now, our porosity measurements are based on unit surface areas and, other things being equal, porosity values for the three groups should be approximately equal. In our experimental groups this is only partially displayed as the variations in the comparatively small groups sampled are sufficiently great to make it difficult to demonstrate this point. It will make the subsequent discussion much simpler if we combine the values for the "large" and "medium" eggs into common or "average" group, and compare these with values for the "small" eggs. The values for the porosity coefficients thus become "small" eggs $5{\cdot}68 \pm 0{\cdot}179$; "average" eggs $5{\cdot}44 \pm 0{\cdot}209$ and within the permissible limits of the standard error these two values are equivalent.

On the other hand, as the "small" egg has a greater surface in proportion to its weight it would be expected the percentage loss (i.e. loss per unit of weight) to be greater than for "average" eggs. Actually we find that the percentage loss for the two types is $0{\cdot}7126 \pm 0{\cdot}023$ for the "small", and $0{\cdot}6078 \pm 0{\cdot}0175$ for the "average", which is what was anticipated. Proceeding to a fuller study of the influence of surface area: the average area of the "small" $= 66{\cdot}8$ cm.$^2$; of "average" $80{\cdot}4$ cm.$^2$, the corresponding values for the respective volumes being $50{\cdot}5$ and $68$ cm.$^3$, therefore the ratio $\frac{\text{area}}{\text{volume}}$ for "small" $= \frac{66{\cdot}8}{50{\cdot}5} = 1{\cdot}31$, and for "average" $\frac{80{\cdot}4}{68} = 1{\cdot}17$, this latter ratio being, of course, the smaller.

Now, while the porosity measurement makes allowance for this

discrepancy in as far as they are calculated for unit surface area the percentage losses do not. Hence $\frac{\text{percent. loss small}}{\text{percent. loss average}} \times \frac{1{\cdot}17}{1{\cdot}31} = 1{\cdot}045$ should give the same result as $\frac{\text{porosity small}}{\text{porosity large}} = 1{\cdot}045$. The agreement is good and it must be obvious that the connexion between porosity values and percentage losses is purely a mechanical one. The only factor which might influence this absolute relationship is the slight variations in specific gravity of eggs as the ratio $\frac{\text{surface area}}{\text{weight}} \times 100$ derives from the ratio $\frac{\text{surface area}}{\text{volume} \times \text{specific gravity}} \times 100$. This factor is automatically adjusted by our calculation. This phenomenon has also been described by Atwood (1901) aud Dunn (1923*a*).

*Influence of thickness.* Next take the influence of thickuess of shell on porosity figures. The values for our two groups are

| | Porosity | Thickness |
|---|---|---|
| "Small" | 5·68 | 0·0128 in. |
| "Average" | 5·44 | 0·0135 in. |

We have already indicated that it is to be anticipated that porosity will vary inversely as shell thickness and if the differences in porosity observed are to be accounted for by this factor then $\frac{5{\cdot}68}{5{\cdot}44}$, which equals 1·045 when multiplied hy $\frac{0{\cdot}0128}{0{\cdot}0135}$ should approach to unity and does in fact equal 0·992. As, however, the observed differences are small and within the range of the standard error, this result must be confirmed if it is to be shown that it is not accidental. The coefficient of correlation between the shell thickness and porosity values of all the brown eggs was therefore calculated and found to equal $-0{\cdot}1758$. This is about the value reported by Bryant & Sharp (1934), and it is clear that thickness has a certain though not very marked influence on porosity values.

## CUTICULAR EVAPORATION

*Method of approach.* In essence the problem is simple. Free albumen evaporates at a certain rate, but, when this is surrounded by shell, the rate is greatly reduced and becomes, in fact, the rate at which an egg loses weight. This reduction has resulted rom the interposition between the albumen and the atmosphere of the egg-shell and its associated structures, viz. the two lining membranes and cuticle. Discount for a

moment the possible influence of these latter. The shell, apart from its pores, is quite impervious to moisture and evaporation takes place only via the pores. The evaporating surface of the albumen has been reduced in the ratio $\frac{\text{area of pores}}{\text{area of shell}}$ and if the area of the pores can be regarded as the true evaporation area then, approximately,

$$\frac{\text{rate of evaporation of egg}}{\text{rate of evaporation of free albumen}} \text{ should equal } \frac{\text{area pores}}{\text{area shell}}.$$

*Albumen.* The "porosity coefficient" of albumen can be determined by methods similar to those given above. A weighed quantity is placed in a dish of known cross-section, the whole placed in the desiccator and incubator and the loss in weight per cm.$^2$ in 24 hr. calculated.

The average value for fifteen determinations was $127 \pm 41{\cdot}5$ mg. cm.$^2$ 24 hr. 37° C.

It will be seen that the readings were rather variable, the extremes noted being 76 and 210 respectively.

If the average value of the porosity for the egg is taken as 5·5, then the shell, etc. has reduced the evaporation rate from 127 to 5·5, and the evaporation has been reduced to $\frac{5{\cdot}5}{127} \times 100 = 4{\cdot}35$ per cent of its initial value.

*Pore area.* The pore area is the area of a single average pore multiplied by the number of pores present in the shell. We take the value of the diameter given by Stewart (1936) as 0·038 – 0·054 mm. This gives an average area for a single pore of $(0{\cdot}0046)^2 \times \frac{\pi}{4} = 1{\cdot}66 \times 10^{-5}$ cm.$^2$

*Number of pores.* Eggs were treated by Almquist's modification of Rizzo's method and the average of several counts through a sq. cm. template taken (Almquist & Holst, 1931). The counts were fairly consistent, a specimen series being 78, 75, 72, 64, 56, 62, average 69·5.

Hence the area of pores per sq. cm. becomes $1{\cdot}66 \times 10^{-5}$ cm.$^2 \times 69{\cdot}5 =$ 0·001154 cm.$^2$ or 0·1154 per cent.

Thus the original tentative supposition that the pore exits might represent the evaporating surface must be erroneous as it will not nearly account for the observed porosity values. In fact they would give a value which is $\frac{4{\cdot}35 \text{ per cent}}{0{\cdot}1154 \text{ per cent}} = 37{\cdot}7$ times too small. A much larger evaporating surface must therefore be looked for.

*Note.* Although it is tacitly assumed that the pores are responsible for the porosity of shells, partly on account of the general obviousness of such a supposition and partly

because there exists a high positive correlation between the pore count and rate of loss of weight on incubation or storage, nevertheless it was felt that a more direct check on this point was advisable. Two identical "artificial eggs" were therefore constructed, the nature of which will be gathered sufficiently from the diagrams. The initial enclosure of the albumen within an inner tube is merely to facilitate the manipulative process of affixing the shell.

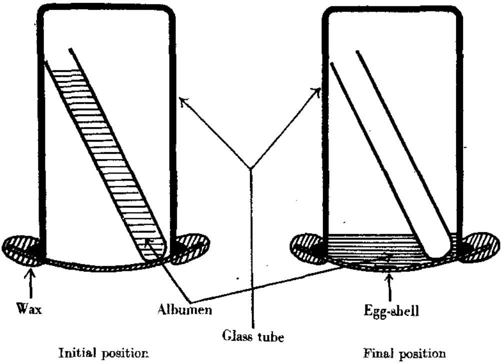


Fig. 3. Artificial egg.

An egg which had been stained by Rizzo's method was cracked open and a piece of shell practically devoid of pores mounted as artificial egg no. 1 and another piece of the same shell containing numerous pores mounted as artificial egg no. 2. The membranes were left intact and the fractured edges carefully sealed with wax and varnish, so that exactly equal areas of the two pieces of shell were exposed. The artificial eggs were then placed in the incubator, and after the lapse of an interval to permit stabilization of pressures and the detection of any leaks, they were removed and weighed, replaced for about 20 hr. and re-weighed. The artificial egg with the numerous pores lost 7·33 times as much moisture as the other with relatively few pores. This seems fairly conclusive evidence of the significance of the pores in relation to moisture losses.

*Cuticular stained areas.* Some pieces of egg-shell were dried and then alcoholic solution of methylene blue applied to the inner surface. It penetrated through the pores and stained the cuticle surrounding the pore exits. To measure the diameter of these areas several pieces of the shell were photographed, the photograph enlarged and the resulting images, which averaged about 1·5 mm. in diameter, measured with a proportion compasses giving a ratio of 1 : 10. The total magnification

thus obtained was $\times 92{\cdot}8$. The average diameter of 137 measured spots, this being the total number of spots on about 2 cm.$^2$ of shell, was 0·0165 cm.

Taking the average pore count per cm.$^2$ as before as 69·5, the area of stained cuticle per $\text{cm.}^2 = (0{\cdot}0165)^2 \times \frac{\pi}{4} \times 69{\cdot}5 = 0{\cdot}01482\ \text{cm.}^2$ or about 1·482 per cent of surface area.

This is much nearer to the 4·35 % observed and is of the right order of magnitude though still $\frac{4{\cdot}35 \text{ per cent}}{1{\cdot}482 \text{ per cent}}$ or 2·94 times too small. The residual discrepancy may be due to some or all of the following causes.

(1) *The extreme variability of albumen porosity, i.e. the average* value used in the calculation may not represent a true value.

(2) Pores vary in diameter; therefore the rate at which the cuticle is supplied with moisture through these varying pores must differ considerably.

(3) The influence of the membrane may be positive in assisting porosity.

DISCUSSION

The part which the cuticular areas described as "plaques" play in the process of evaporation from the egg is thus manifestly clear. It appears that their presence is vital to the developing chick, for in their absence evaporation would be so greatly reduced that it is unlikely that under average incubation conditions the air space would ever exceed 1/40 of that required for ideal hatching, viz. $\frac{15}{40}$ per cent or 0·375 per cent.

But can these plaques be regarded as cuticular structures? This is a point which the authors are not prepared to affirm, but if the cuticle be regarded as the non-calcareous external covering of the shell then these orificial plaques must be included in such a definition. That they do differ in some respects from the general cuticle is quite certain; they are not mere artefacts arising from the passage of dye into the cuticle, similar, say, to ink spots on a piece of blotting-paper. Their edges are much too sharply defined and depart considerably from the circular form which such an origin would demand. Moreover, if an unbroken egg is immersed in dye, removed and rinsed, the plaques are quite sharply stained; this is the most conclusive evidence of their individuality, as otherwise the whole cuticle would be equally stained by this procedure. On the other hand, it must be admitted that these plaques are well protected in conntersunk depressions, and that "washing" of the egg does not remove them. It may well be that the divided opinion held regarding the nature

of the cuticle, a fact already alluded to, arises from a misconception of its dual structure.

In the light of the investigation described above it seems that a detailed study of the interrelationships of the pores, pore contents, cuticle and "plaques" might be a subject of considerable interest to poultry science.

## SUMMARY

1. The porosity of egg is measured by the usual practice of estimating the weight loss under standard conditions. The porosity coefficient is expressed as the loss in weight in mg. per sq. cm. of egg surface per 24 hr. at 37° C.

$$P \text{ coeff.} = \text{mg. cm.}^2 \text{ 24 hr. } 37°.$$

2. It is shown that eggs of even curvature approximate closely to the ideal mathematical form of two hemi-prolate spheroids, differing in their eccentricities and meeting in the plane of their common minor axes. On this assumption the divergence between the observed and calculated long perimeter is only +0·618 per cent (five eggs) and between the observed and calculated volumes + 3·78 per cent (seventy eggs).

3. It is explained how, by taking the specific gravity of the egg to correct for departures from the theoretical form, it is possible to calculate the surface area with an error not exceeding 1 per cent.

4. Comparisons are next made of the porosity coefficients of several groups of eggs (about sixteen in each group) and it is shown that

(*a*) Brown eggs are less porous than white (4·8637 ± 0·177 compared with 6·1125 ± 0·87).

(*b*) Small eggs lose weight more rapidly than large eggs due to their relatively greater evaporating surface (0·7126 ± 0·023 per cent compared with 0·6078 ± 0·0175 per cent).

(*c*) Thickness of the shell has a definite though inconsequential influence on porosity, thick shells tending to lower porosity (coefficient of correlation − 0·1758).

5. The main point of the paper is next considered. Evaporation from an egg is only 4·35 per cent of that from free albumen. This decrease arises from the reduction of the evaporating surface by the interposition of the shell. If the pore orifices are regarded as the new evaporating surface this will account for an evaporation of only 0·1154 per cent. If the cuticular areas immediately surrounding the pore exits are considered as the evaporating surface (areas which are well defined and stain readily by dyes penetrating from within the egg) this will explain an evaporation of 1·482 per cent.

This is still some 2·94 times too small $\left(\frac{4\cdot35 \text{ per cent}}{1\cdot482 \text{ per cent}}\right)$ but is of the order of magnitude of the observed values. Possible explanations of the residual discrepancy are mentioned.

6. In view of the significance of the orificial plaques to the vital functions of shell porosity attention is drawn to the desirability for a fuller study of their structure, and more especially of the precise nature of their relationship to the organic contents of the pore.

ACKNOWLEDGEMENT

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## DESCRIPTION OF PLATE I

In all specimens dye introduced into the inside of the egg has penetrated outwards via the pores and stained the surface plaque.

Figs. 1 and 2. Cross-section of shell showing pore and surface plaque lying in recessed area of shell and stretching over pore mouth. Note intense staining of lining membrane. The inner ends of both pores are occupied by a single mammilla.

Fig. 3. Surface of shell showing distribution of plaques.

Fig. 4. Enlarged surface view of plaques. Note irregular outline and sharp margins.

Fig. 5. Dye allowed to act for shorter period. Note that it is the marginal region which is first stained, the dye penetrating the plaque from the edges inwards. The central region shows radiating fissures. These result from the "drying" of the plaque.

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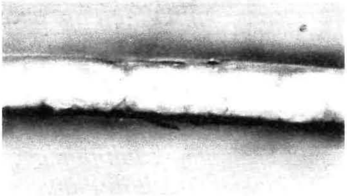

Fig. 1.

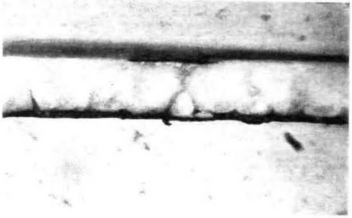

Fig. 2.

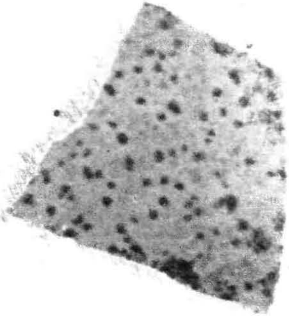

Fig. 3.

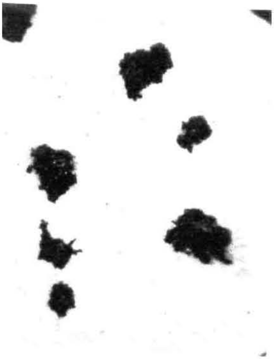

Fig. 4.

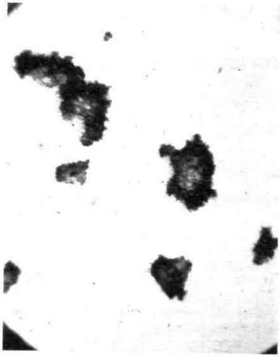

Fig. 5.

# THE MECHANISM OF CELLULOSE DIGESTION IN THE RUMINANT ORGANISM

## IV. FURTHER OBSERVATIONS FROM *IN VITRO* STUDIES OF THE BEHAVIOUR OF RUMEN BACTERIA AND THEIR BEARING ON THE PROBLEM OF THE NUTRITIVE VALUE OF CELLULOSE

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### Introduction

The results of investigations into the mode of action of thermophilic cellulose-splitting bacteria have been described in previous communications (Woodman, 1927, 1930; Woodman & Stewart, 1928, 1932). It was shown that the normal end-products of fermentation of pulped filter paper at 65° C. were organic acids and gaseous compounds, but that when an antiseptic such as toluene was shaken into the medium at the "head" stage of the reaction and the incubation continued at 37° C., the production of glucose could be demonstrated without difficulty. This finding was interpreted as showing that glucose is an intermediate phase in the breakdown of cellulose by bacteria.

Since no further breakdown of cellulose appeared to take place in the second stage of incubation following the addition of toluene, it was concluded that the glucose was being produced from partially hydrolysed cellulose, and that the toluene had caused the inactivation of the bacterial enzymes responsible for the initial stage of cellulose hydrolysis. This explanation would account for the small yields of glucose obtained.

It was noted in the course of this work that filter paper torn by hand was fermented much more readily than filter paper cut by scissors. Obviously the frayed edges of the hand-torn filter paper were more open to attack by the micro-organisms than the clean edges of the scissors-cut filter paper. For the purposes of the present fermentation studies, coarsely torn filter paper was reduced to a pulp with boiling water, filtered off and dried in a steam oven. The dried material was finally reduced to an extremely fine state of division by means of an electric mill.

THE TEMPERATURE OF THE FERMENTATION REACTION

The cultures of cellulose-splitting bacteria used in the investigations referred to above were obtained by incubating at 65° C. material from an old manure heap rich in hot, actively fermenting horse dung. The incubation was carried out in a medium containing, per 100 c.c. tap water, 2 g. filter paper, 1·2 g. calcium carbonate, 0·5 g. sodium phosphate, 0·25 g. ammonium sulphate and 0·1 g. potassium chloride. The micro-organism grew quite satisfactorily in the absence of organic nitrogen, but after making the 106th subculture, it began to display a rapidly waning power to ferment cellulose. The discovery was made at this stage that the addition of a little organic nitrogen, in the form of casein, had the effect of renewing the fermentative power of the culture. In subsequent work a small "pinch" of casein was always added to the subcultures.

The micro-organism cultured from the fermenting horse dung was thermophilic, its optimum temperature of fermentation being in the neighbourhood of 65° C. Attempts to grow the bacteria at 37° C. were unsuccessful, a somewhat disconcerting finding in view of the fact that bacterial fermentation of cellulose in the rumen must necessarily take place at a temperature of about 40° C.

Further work carried out by the present writers has made the position clearer. It has now been found that if a small amount of the rumen contents of a sheep be incubated at 37° C. with shredded filter paper in the usual medium, fermentation takes place initially more readily at this temperature than at 65° C. Nor is any difficulty experienced in preserving the activity of the micro-organism by continuous subculturing at 37° C. By the addition of toluene at the "head" stage, it is possible, moreover, to demonstrate, as with the thermophilic bacteria, the production of a small amount of glucose during subsequent incubation. The earlier finding may be explained as follows: (1) the conditions in hot, fermenting horse dung, from which the first cultures were made, were such as to favour the growth of the thermophilic bacteria and to inactivate the micro-organisms capable of fermenting cellulose at the lower temperature; (2) continuous subculturing at 65° C., beginning with either horse dung or rumen contents, results in the inactivation of all forms save the thermophilic bacteria. It may be observed at this point that cultures of bacteria capable of fermenting cellulose at 37° C. were readily obtained from fresh sheep dung, pointing to the probability of cellulose breakdown in the intestine as well as the rumen.

Since the earlier studies had been restricted to cellulose fermentation

at 65° C. by thermophilic bacteria, it was clearly desirable to undertake comparative investigations to ascertain whether the course of the reaction, and the end-products therefrom, were altered when the fermentation was brought about at 37° C. The findings of these further inquiries are brought forward in the present paper. It should be pointed out that no attempt has been made in this work to obtain pure cultures by prolonged and continuous subculturing, since it was desired to carry out the experiments under conditions as comparable as possible with those obtaining in the rumen itself.

### THE NATURE OF THE VOLATILE FATTY ACIDS PRODUCED IN CELLULOSE FERMENTATION

A detailed investigation was made of the nature of the volatile fatty acids produced by bacterial fermentation of the prepared filter paper, both at 65 and 37° C. The determinations were carried out after fermentation had proceeded for some days and almost the whole of the filter paper had disappeared. For this purpose Dyer's modification (1916–17) of the well-known Duclaux method was used, confirmation of the conclusions thus made being obtained by specific chemical tests.

From the standpoint of production of volatile fatty acids, no striking differences were noted between the reactions as carried out at 65 and 37° C. The nature and proportions of the acids, however, appeared to vary considerably from culture to culture. Thus, with one culture of rumen bacteria (65° C.), the fermentation of 10 g. filter paper gave rise to 1·7 g. butyric acid and 1·3 g. formic acid, with only a trace of acetic acid. In another case, working under the same conditions, the fatty acid produced was almost exclusively acetic acid (3·2 g.) with traces only of butyric and formic acid. Findings of an equally inconsistent nature were obtained with cultures of rumen bacteria at 37° C.

The investigations of Tarvin & Buswell (1934) into the nature of the bacterial action in sludge digestion tanks suggest that the fatty acids arising during bacterial breakdown undergo decomposition with the production of formic acid and hydrogen

$$(RCH_2COOH + 2H_2O = RCOOH + HCOOH + 2H_2).$$

This is followed by decarboxylation with the formation of $CO_2$, the latter then reacting with the hydrogen to give methane. If this theory be accepted, it is clear that the study of the quantitative aspects of the production of volatile fatty acids during fermentation *in vitro* may lead to misleading conclusions, since the nature and amounts of such acids in

the medium will be dependent on the extent to which these secondary changes have occurred at the time of the determinations.

During the steam distillation of the volatile organic acids, a small amount of white, flaky material was observed to form in the tube of the condenser, suggesting the presence of a higher fatty acid. But although this separation was noted with great consistency, the amount was so small that it was found impossible to isolate the flaky substance in sufficient quantity for the purpose of chemical tests.

It might be argued that these findings in respect of production of volatile fatty acids cannot with justice be held to give a true picture of what might occur in the ruminant digestive tract, in that the conditions of the rumen would probably be more completely anaerobic than was the case in these *in vitro* experiments. To test this point, further experiments were made in which the medium was covered with a layer of liquid paraffin, whilst in other cases nitrogen was used to replace air. Fermentation occurred readily under these conditions, but no essential differences were noted in regard to the nature of the volatile fatty acids produced.

From the standpoint of fat production in the animal, it would appear that the volatile fatty acids arising from the bacterial fermentation of cellulose have little significance. The bulk of evidence at the present time rules out formic, acetic and butyric acids as possible glucose or fat precursors (Woodman, 1930). Ringer & Jonas (1922) state that of the lower normal fatty acids, only propionic, valeric and heptylic acids are glucose precursors, but not formic, butyric and caproic acids. Eckstein (1933) administered propionic, butyric, valeric and caproic acids to rats and found that, of these acids, only propionic acid was able to lead to glycogen formation in the liver. The possibility of acetic and butyric acids being converted into glycogen in the liver has been re-examined recently by Deuel *et al.* (1935–6). The conclusion was reached that the even carbon-chained fatty acids do not produce glycogen. This was further supported by their work on phlorizinized animals, no glucose appearing in the urine after administration of either sodium acetate or butyrate, whereas propionic and lactic acids were converted quantitatively into glucose under these conditions. Of the simple fatty acids, therefore, it may be concluded that only propionic acid is able to function as a glucose or fat precursor, but it will be noted that in the present work on the bacterial fermentation *in vitro* of cellulose, propionic acid, if formed at all, was present in such small amount as to escape detection.

The results of the present investigation in regard to the production

of volatile fatty acids as important end-products of the bacterial decomposition of cellulose are substantially in harmony with those of other workers (Viljoen *et al.* 1926) and apparently afford no clue to the manner in which cellulose is utilized for fat production in the ruminant. In an attempt to reconcile the theory of cellulose digestion with Kellner's finding (1905) that digested cellulose and starch have equal fat-forming powers in the ruminant, Woodman (1927) put forward the tentative hypothesis that the bacterial digestion of cellulose in the rumen might be so controlled as to give rise to glucose, with cellobiose as an intermediate phase, and that, as with starch, about 10 % of the energy in the cellulose might be lost as a consequence of destructive fermentation to methane. In support of this hypothesis, Woodman & Stewart (1928) demonstrated the possibility of glucose production from cellulose in artificial media by exercising control over the activity of the cellulose-splitting micro-organisms.

In the light of further studies, both in artificial media and with sheep in which rumen fistulas had been established, this hypothesis was quickly abandoned (Woodman & Evans, 1936). It was concluded that although glucose is undoubtedly an intermediate phase in cellulose fermentation, the quick breakdown which it undergoes precludes the possibility of its forming a main end-product in cellulose digestion in the animal. If cellulose reaches the blood stream in the form of glucose at all, this can only happen to a very limited degree.

It became necessary, therefore, to pay greater attention to the possible formation, during cellulose fermentation, of such recognized glucose and fat precursors as lactic and pyruvic acids. A study of the literature dealing with the bacterial breakdown of cellulose leads to the belief that, from the standpoint of accounting for the value of this constituent in nutrition, too much importance has been attached to the study of the *end-products* of fermentation and to the drawing up of carbon and energy balances on this basis. Since absorption from the digestive tract is proceeding continuously, it is clearly of primary significance to investigate in detail the whole course of the reaction and to study the nature, and behaviour during fermentation, of any intermediate products that may arise, even though in but minute amount. It will be shown later that a simple examination of the end-products of the reaction in artificial media may give a totally inadequate picture of what may be taking place in the digestive tract of the animal.

With this idea in mind, a study has been made in this work of the changes undergone by the intermediate products of cellulose fermentation

under the influence of rumen bacteria. Since glucose has been shown to be one of the earliest phases to arise in the breakdown of the cellulose molecule, it was felt in particular that an investigation of the behaviour of this hexose under the influence of rumen bacteria might shed further light on the manner in which cellulose is utilized by ruminants for fat production.

### The action of rumen bacteria on glucose at 37° C. The production of lactic and pyruvic acids

In the experiments to be described, the $CaCO_3$, the glucose solution and the culture medium (see earlier section of this paper) were sterilized separately before admixture. It was found, as might be anticipated, that the rumen bacteria are able to ferment glucose much more readily than cellulose. Thus, the addition of glucose to culture media containing cellulose leads to the reaching of the "head" stage in about 12 hr. instead of 2–4 days when only cellulose is present. Where quantitative results are cited in this account, it may be assumed that the experiments in question have been repeated a number of times to secure the necessary confirmation.

Lactic acid was determined by the method of Friedemann *et al.* (1927, 1929), precautions being taken to remove, by brief aeration of the boiling liquid before addition of colloidal $MnO_2$, any traces of acetaldehyde or ethyl alcohol that might have been formed during fermentation. It may be noted, however, that no evidence was obtained of the actual presence of either of these compounds. No quantitative determinations of pyruvic acid were made, its presence (or absence) being shown by the nitroprusside reaction of Simon & Piaux (1924, see also Case & Cook, 1931), the intensity of the blue colour giving a good indication of the concentration of pyruvic acid at any given stage of the fermentation. Confirmation of the presence of pyruvic acid was obtained by isolating the characteristic hydrazone with 2 : 4-dinitro-phenylhydrazine. Special tests were made to ensure that the presence of pyruvic acid was not dependent on the "pinch" of casein that was always added to the medium. No fixing agent was necessary in order to demonstrate the formation of pyruvic acid, probably because of the presence of excess $CaCO_3$ in the medium. Fernbach (1913) has noted that in the case of yeast fermentation, the maintenance of an alkaline reaction by the introduction of chalk serves to check the disappearance of pyruvic acid sufficiently to enable its presence to be detected.

200 c.c. of the culture medium (containing twice the usual concen-

tration of salts, but no cellulose) and 200 c.c. of a solution of glucose of roughly 5 % strength were sterilized separately and mixed after cooling. To the mixture were added 5 g. of sterilized $CaCO_3$ and a "pinch" of casein; 50 c.c. of a culture of rumen bacteria (9th subculture from rumen contents of sheep, the incubations having been carried out at 37° C. in the presence of shredded filter paper) were then run in. The flask with its contents was placed in an incubator at 37° C. and was shaken at occasional intervals.

After 24 hr. the flask was well shaken and 20 c.c. of the contents withdrawn and centrifuged. Determinations of glucose (Bertrand's method), lactic acid, volatile fatty acids, etc., were made on the clear liquid, glucose being removed by the $CuSO_4$-$Ca(OH)_2$ reagent before proceeding with the estimation of the lactic acid. Analytical determinations were made daily in this manner up to day 8, and the results are shown in Table I. The intensity of the reaction for pyruvic acid is shown by symbols, the symbol + + +, for example, denoting a very strong colour reaction.

Table I. *Showing course of fermentation of glucose by rumen bacteria at 37° C.*

| Incubation period days | Amounts per 100 c.c. medium: Glucose g. | Lactic acid g. | Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
|---|---|---|---|---|---|
| 1 | 2·14 | Trace* | Trace* | Negative | Slight gas production |
| 2 | 1·90 | ,, | 0·14 | + + + | ,, |
| 3 | 1·56 | 0·03 | 0·24 | + + + | Fermentation becoming active. Strong smell of $H_2S$ |
| 4 | 0·95 | 0·34 | 0·28 | + + | Copious gas production. Smell of $H_2S$ still strong. Solution becoming turbid† |
| 5 | Trace | 1·07 | 0·32 | + + | Copious gas evolution. Only trace of $H_2S$ discernible. Medium quite cloudy |
| 6 | — | 1·13 | 0·32 | + | Very little gas production |
| 7 | — | 0·69 | 0·45 | Negative | Gas production again copious |
| 8 | — | 0·02 | 0·58 | ,, | Gas production still copious |

* It should be kept in mind that traces of organic acids are introduced into the medium when inoculating from previous subcultures.

† Shown to be due to presence of $CaSO_4$.

The results in Table I show that pyruvic acid is produced at a relatively early stage of the fermentation, the maximum reaction for this acid being obtained before it was possible to demonstrate the presence of more than a trace of lactic acid. After the third day the amount of pyruvic acid declined rapidly, during which time (days 4, 5 and 6) the

concentration of lactic acid increased steeply to a maximum of 1·13 % on day 6. At this stage, gas production, which had been very copious on days 4 and 5, had almost ceased, and the strong smell of $H_2S$, which was noted on days 3 and 4, had almost disappeared.

Gas production became pronounced again on day 7. Pyruvic acid and glucose were now absent from the medium, and the concentration of lactic acid began to fall rapidly, accompanied by a sharp rise in the amount of volatile fatty acids. On day 8 only a trace of lactic acid remained in the medium.

The results point to the conclusion that during the fermentation of glucose at 37° C. by cultures of rumen bacteria, pyruvic acid is first formed, and that this may then undergo reduction to lactic acid. This aspect of the changes will be referred to again at a later stage of this section. At the same time, side reactions are taking place whereby lactic acid, and probably pyruvic acid also, are being transformed into volatile fatty acids and gases. These findings are in harmony with the recent work of Wood *et al.* (1937) in which evidence was obtained supporting the intermediate nature of phosphoglyceric, pyruvic, lactic, acetic and succinic acids in the fermentation of glucose by the propionic bacteria.

The course of the fermentation in the experiment just described, particularly in the early stages, was somewhat more sluggish than was noted in other experiments where inoculation of the medium was effected directly by the addition of the liquid (50 c.c. per 500 c.c. medium) squeezed out from a portion of the rumen contents of a sheep subsisting on young leafy pasturage. In one such experiment, for example, the whole of the glucose had disappeared by the third day of incubation at 37° C., when the medium contained 0·86 % of lactic acid and 0·35 % of volatile fatty acids (expressed in terms of acetic acid). The concentration of lactic acid had fallen on day 6 to 0·01 % with a rise in the volatile fatty acids to 0·8 %.

It is clear, therefore, that in the early stages of the fermentation, considerable amounts of lactic acid are produced from glucose by the rumen bacteria, and further, that the lactic acid accumulates more rapidly than the volatile fatty acids. In the later stages, however, the lactic acid declines in amount and ultimately disappears from the medium, whereas the lower fatty acids rise during this phase to a maximum, a change accompanied by considerable gas evolution.

From the standpoint of the animal, it is obvious that erroneous conclusions might be drawn by relying on an examination of the end-products of the fermentation rather than on the investigation of the

character and amount of the intermediate products. If the results of these *in vitro* experiments have any justifiable application to the animal, it is probable that the main breakdown products being absorbed from the tract during the digestion of cellulose would be the fat precursors, lactic and pyruvic acids, rather than the volatile fatty acids. This statement, of course, implies that cellulose passes, by hydrolysis, through the glucose stage before undergoing further breakdown.

In view of the disappearance of lactic acid in the second stage of the fermentation, experiments were carried out in which glucose was replaced by lactic acid and sodium lactate as the source of assimilable carbon. To 100 c.c. of the usual medium (containing 3 instead of 1·2 g. $CaCO_3$) was added 1·25 g. lactic acid. After sterilization, the contents of the flask were inoculated with 20 c.c. of a subculture of rumen bacteria and placed in an incubator at 37° C. for 3 weeks. After this time the medium contained only a trace of lactic acid, while the volatile fatty acids (mainly acetic acid) amounted to 88 c.c. $N/10$.

In another experiment a sterilized solution of sodium lactate was added to a medium containing actively fermenting filter paper. After 48 hr. incubation at 37° C., the concentration of lactic acid fell from 334 to 28 mg. per 100 c.c. Further sodium lactate was now added to the medium and the incubation continued for 17 days. During this time almost the whole of the lactic acid disappeared, 0·01 g. only remaining from the 1·12 g. added in the form of sodium lactate. The volatile fatty acid produced (0·45 g. as acetic acid) consisted mainly of acetic acid with a trace of butyric acid.

The foregoing observations confirm the conclusion derived from the experiments with glucose that lactic acid is readily broken down by the rumen bacteria to gaseous products and volatile fatty acids, of which acetic acid appears to be present in highest amount. It is particularly unstable when it constitutes the sole source of assimilable carbon.

In discussing the fermentation of glucose by the rumen bacteria, the results in Table I have been taken as warranting the conclusion that pyruvic acid is to be regarded as a precursor of lactic acid, a conclusion in harmony with the work of Nelson & Werkman (1935) who propound the scheme that 2 molecules of pyruvic acid give rise to 1 molecule of lactic acid, 1 molecule of acetic acid and 1 molecule of $CO_2$. Experiments were next conducted, therefore, to determine the nature of the products arising from the action of rumen bacteria on pyruvic acid when the latter constitutes the sole source of assimilable carbon in the medium.

400 c.c. of the usual culture medium (containing 8 g. $CaCO_3$) was

sterilized and, after cooling, thoroughly shaken with 10 c.c. pyruvic acid. After standing for 48 hr., 5 c.c. of rumen liquid and a "pinch" of casein were added. Determinations of lactic, pyruvic and volatile fatty acids were carried out, and the flask with its remaining contents was then placed in an incubator at 37° C. Further determinations were made at daily intervals, and the results are tabulated in Table II.

Table II. *Showing course of fermentation of pyruvic acid by rumen bacteria at 37° C.*

| Incubation period days | Amounts per 100 c.c. medium: Pyruvic acid g. | Lactic acid g. | Vol. fatty acids as acetic acid g. | Remarks |
|---|---|---|---|---|
| 0 | 1·17 | 0·18 | 0·17 | — |
| 1 | 0·46 | 0·20 | 0·34 | Vigorous gas production. No smell of $H_2S$ |
| 2 | — | 0·16 | 0·83 | ,, ,, |
| 3 | — | 0·12 | 1·04 | Slight gas production. No smell of $H_2S$ |

Lactic acid was determined by the method of Friedemann *et al.* (1927). These workers state that pyruvic acid has little or no disturbing influence on the determination of lactic acid, a claim which is supported by Stewart *et al.* (1934), who found that among the possible precursors of lactic acid, methyl glyoxal alone interferes with the determination. Pyruvic acid was determined by the method of Wendel (1931–2). In the estimation of the volatile fatty acids, the first distillate was submitted to redistillation to eliminate possible traces of pyruvic acid. For this purpose it was necessary to reduce the original distillate to a small bulk by evaporation under slightly alkaline conditions.

Although the writers do not claim to be entirely satisfied about the absolute reliability of the analytical methods employed in this experiment, it is clear from the results in Table II that pyruvic acid, when constituting the sole source of assimilation carbon, is readily fermented by the rumen bacteria. There is no evidence that lactic acid is produced during these changes, but rather that the reaction gives rise to the production of volatile fatty acids and gases, for example

$$CH_3COCOOH + H_2O = CH_3COOH + H_2 + CO_2.$$

The possibility must be kept in mind, however, that any lactic acid that might arise from pyruvic acid under these conditions may undergo such rapid conversion into gases and volatile fatty acids as to escape detection by the analytical methods employed.

The peculiar observation was made that the medium still gave the

blue colour of the Simon & Piaux test for pyruvic acid for some days after analysis had indicated the apparent entire disappearance of this acid. It is probable, therefore, that the sample of pyruvic acid used in the experiment contained a condensation product of pyruvic acid which did not reduce to lactic acid by the action of the Zn-Cu reagent in the determination of pyruvic acid by Wendel's method. Clift & Cook (1932) state that pyruvic acid undergoes spontaneous conversion to $\alpha$-keto-$\gamma$-valerolactone-$\gamma$-carboxylic acid, and that commercial samples of pyruvic acid may contain up to 30 % of this condensation product. Quastel & Wooldridge (1929) observed that when a neutral pyruvate is incubated for long periods with *B. coli*, a substance is formed which, on alkaline hydrolysis, reverts to pyruvic acid. It may be that the gradual breakdown of the condensation product during incubation must be held responsible for the rises in the amount of volatile fatty acids during the later stages of the fermentation. The presence of some such complex polymer after 72 hr. incubation was shown by boiling some of the medium with dilute $H_2SO_4$, filtering and, after making strongly alkaline, boiling for a few minutes with Fehling's reagent, when a fairly copious separation of cuprous oxide was noted.

The foregoing experiment appears to lead to the conclusion that the disappearance of the pyruvic acid which arises during the first stage of the glucose fermentation (see Table I) may not be due to its reduction to lactic acid, but to its breakdown to volatile fatty acids and gases. The lactic acid, therefore, may not arise directly from pyruvic acid, but may be formed in an independent reaction. The experiment, however, cannot be considered conclusive on this point, since the behaviour of pyruvic acid arising as an unstable intermediate phase in the presence of actively fermenting glucose may conceivably be different from that when pyruvic acid constitutes the sole source of assimilable carbon in the medium. Stephenson (1930) is of the opinion that reduction of pyruvic to lactic acid does not commonly occur, and that most probably the principal route by which pyruvic acid disappears during the course of fermentation is into the substance of the bacterial cell.

## FERMENTATION OF CELLULOSE BY RUMEN BACTERIA AT 37° C.

Since glucose arises as an early but transient phase in the bacterial breakdown of cellulose (Woodman & Stewart, 1928), it was natural to anticipate that the course of fermentation of cellulose by rumen bacteria should display features in common with those noted in the bacterial fermentation of glucose. That lactic acid is formed during cellulose

fermentation was shown by the thiophene reaction and by the isolation of small amounts, in the form of crystalline zinc lactate, from media containing fermenting filter paper.

450 c.c. of the usual culture medium (10 g. shredded filter paper) was inoculated by the addition of 50 c.c. of a subculture (No. 6) of rumen bacteria, the customary precautions being taken. The analytical results obtained during the period of incubation at 37° C. are shown in Table III.

Table III. *Showing course of fermentation of cellulose in prepared filter paper at 37° C.*

| Incubation period days | Amounts per 100 c.c. medium: Lactic acid mg. | Amounts per 100 c.c. medium: Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
|---|---|---|---|---|
| 3 | 6·8 | 0·24 | + + + | Gas production becoming vigorous. Strong smell of $H_2S$ |
| 4 | 7·2 | 0·26 | + + + + | "Head" stage |
| 5 | 20·4 | 0·34 | + + + | Copious gas production |
| 6 | 35·3 | 0·38 | + | ,, |
| 7 | 35·6 | 0·45 | Faint | ,, |
| 8 | 21·3 | 0·48 | Negative | ,, |
| 9 | 6·3 | 0·50 | ,, | ,, |
| 10 | 6·0 | 0·51 | ,, | Fermentation still active with small amount of filter paper remaining. $H_2S$ still evident |

It will be noted from the results in Table III that, in a qualitative sense, the fermentation of cellulose at 37° C. takes a course similar to that already described for glucose. Strong positive tests for pyruvic acid were obtained in the first half of the incubation. After day 6, however, the nitroprusside reaction gave a negative response for pyruvic acid and the concentration of lactic acid rose to a maximum on day 7. Subsequently the amount of lactic acid fell sharply, this change being accompanied by a gradual rise in the amount of volatile fatty acids.

From the quantitative standpoint, however, the fermentations of glucose and cellulose at 37° C. were sharply differentiated, the amount of lactic acid produced from the cellulose being extremely small compared with the volatile fatty acids, a finding in marked contrast with that for glucose (see Table I). It is recognized, however, that the importance of an intermediate product is not measured by the actual amount which may accumulate temporarily in the medium. The concentration of lactic acid at any given time will depend on the difference between the rates of its production from its precursor and its further breakdown into gases

and volatile organic acids. In the case of the resistant cellulose, the rate of production of lactic acid at 37° C. is clearly much slower than with the more readily fermented glucose, and this distinction is sufficient to account for the observed quantitative differences in respect of lactic acid accumulation in the two processes. Whether in the animal the pyruvic and lactic acids produced from cellulose in the rumen fermentation are absorbed from the tract sufficiently promptly to obviate their further breakdown into volatile fatty acids is a question that must be left over for discussion until the work on sheep with rumen fistulas is published.

That the cellulose of feeding stuffs behaves similarly during bacterial fermentation to the cellulose in the prepared filter paper is shown by the results in Table IV obtained when 10 g. of crude fibre from wheat straw was used instead of filter paper in a repetition of the experiment summarized in Table III.

Table IV. *Showing course of fermentation at 37° C. of crude fibre from wheat straw*

| | Amounts per 100 c.c. medium | | | |
|---|---|---|---|---|
| Incubation period days | Lactic acid mg. | Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
| 5* | — | — | + + + + | Copious gas production |
| 6* | — | — | + + + + | ,, |
| 7 | 11·1 | 0·35 | + + | ,, |
| 8 | 6·8 | 0·36 | + | ,, |
| 9 | 6·1 | 0·36 | Negative | Slight gas production |

* Lactic acid and volatile fatty acids not determined on these days.

It will be observed from Table IV that the crude-fibre and filter-paper fermentations displayed the same qualitative features, but that the reaction was slower in the case of the fibre. In all probability this is to be ascribed partly to the less favourable physical condition of the fibre and partly to the association of its cellulose with protective lignifying substances.

### FERMENTATION OF CELLULOSE BY THERMOPHILIC RUMEN BACTERIA AT 65° C.

Observations were made in preliminary experiments suggesting that the course of cellulose fermentation by the rumen micro-organisms capable of acting at 65° C. might in its quantitative, though perhaps not

qualitative, aspects be strikingly different from that associated with the bacteria bringing about fermentation at 37° C. In an estimation of the amount of lactic acid in the end-products of fermentation at 65° C., it was found in one experiment that 1·07 g. lactic acid was present per 10 g. filter paper fermented, whereas in two similar experiments conducted at 37° C., the amounts of lactic acid were only 0·16 and 0·02 g. respectively. Although such examination of the end-products constitutes an unreliable basis for elucidating the actual course of the fermentation, it appeared justifiable to assume from these preliminary observations that accumulation of lactic acid during the reaction might be much more considerable at 65 than at 37° C. Further tests showed, moreover, that lactic acid, when added to actively fermenting cellulose in the usual media, disappears much more rapidly at 37 than at 65° C. On the other hand, however, the nitro-prusside colour test for pyruvic acid in the first stage of fermentation was found to be much fainter at 65 than at 37° C., suggesting that this acid represents a more fugitive phase at the higher temperature owing to its readier transformation into other products by the thermophilic bacteria.

A culture of the thermophilic bacteria was made by inoculating the usual medium with rumen liquid, obtained by straining through linen a portion of the very actively fermenting contents of the rumen of a sheep that was subsisting wholly on young, leafy pasturage. 50 c.c. of the fourth subculture at 65° C., in which active gas production was taking place, was added, with the customary precautions, to the usual

Table V. *Showing course of fermentation of the cellulose of prepared filter paper at 65° C.*

| Incubation period days | Amounts per 100 c.c. medium: Lactic acid g. | Amounts per 100 c.c. medium: Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
|---|---|---|---|---|
| 3 | Trace | 0·07 | Trace | Moderate gas production |
| 4 | 0·08 | 0·13 | ,, | Active gas production. Filter paper pigmented* |
| 5 | 0·39 | 0·22 | Negative | "Head" stage |
| 6 | 0·59 | 0·25 | ,, | Gas production slowing down |
| 7 | 0·78 | 0·31 | ,, | Slight gas production |
| 8 | 0·75 | 0·36 | ,, | ,, |
| 10 | 0·64 | 0·48 | ,, | ,, |
| 12 | 0·66 | 0·50 | ,, | No gas evolution. Small amount of filter paper still present |

* In all the fermentation tests carried out at 65° C., the filter paper became yellowish or cream-coloured in consequence of pigment production.

medium (8 g. prepared filter paper), the total volume amounting to 400 c.c. The flask was then placed in an incubator at 65° C., the usual analytical determinations being made at daily intervals during the period of incubation. The results are shown in Table V.

The results in Table V are remarkable for the high amounts of lactic acid that were present in the medium during the fermentation of the filter paper at 65° C., amounts very greatly in excess of those detected when the fermentation was carried out at 37° C. (see Table III) and, indeed, of the same order as were found during the fermentation of glucose at 37° C. (see Table I). If such pronounced lactic acid accumulation had been noted during the fermentation of cellulose at 37° C., the problem of accounting for the nutritive value of cellulose might not be difficult. It would appear, however, that lactic acid breaks down to gases and volatile fatty acids much more readily under the influence of rumen bacteria acting at 37° C. than when the fermentation is brought about by thermophilic rumen bacteria at 65° C. Indeed, lactic acid appears to display a good degree of stability during the incubation at the higher temperature. With pyruvic acid, however, the reverse appears to be the case, since only the very faintest tests for this acid were obtained at the outset of fermentation at 65° C., whereas strong reactions were noted in the first half of the fermentation at 37° C. (compare results in Tables III and V).

A repetition of the foregoing experiment, using the same subculture for purposes of inoculation, gave the confirmatory results shown in Table VI. It should be added that further repetitions in which inoculations were made from a number of different subcultures showed that while lactic acid accumulation at 65° C. was not invariably of such a high order as was noted in the experiments summarized in Tables V and VI, yet in every case it exceeded very considerably that which characterized the fermentation of cellulose at 37° C.

It appears justifiable to conclude from the results in Tables III, V and VI that the fermentations of cellulose at 37 and 65° C. are sharply differentiated in the following respects: (1) at 37° C. pyruvic acid forms a prominent phase during the first half of the reaction, whilst lactic acid displays great instability and is never present in more than small amount; (2) at 65° C. pyruvic acid constitutes an exceedingly fugitive stage, only the weakest reaction for this acid being obtained at the outset of fermentation. Lactic acid, on the other hand, displays a good degree of stability and may accumulate in amounts comparable with those obtained during the fermentation of glucose at 37° C.

Table VI. *Further results showing course of fermentation of cellulose in prepared filter paper at 65° C.*

| Incubation period days | Amounts per 100 c.c. medium: Lactic acid g. | Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
|---|---|---|---|---|
| 3 | Trace | 0·11 | Trace | Copious gas production |
| 4 | 0·25 | 0·21 | Negative | "Head" stage |
| 5 | 0·44 | 0·25 | ,, | Copious gas production |
| 6 | 0·55 | 0·32 | ,, | Moderate gas production |
| 7 | 0·51 | 0·39 | ,, | Slight gas production |

The foregoing conclusions were open to the objection that although the rumen material used for preparing the cultures came from the same sheep, some months separated the carrying out of the fermentation studies at the lower and higher temperatures. It was possible that the character of the microflora in the rumen might be subject to a seasonal variation related to the type of diet on which the sheep was subsisting and, for this reason, the separate studies of the fermentations at 37 and 65° C. might not have been carried out on a comparable basis.

This objection was overcome by preparing cultures of both kinds of micro-organism by inoculations from the same stock of material taken from the rumen of the sheep when subsisting wholly on a diet of young leafy pasturage. The second subcultures were used for comparative studies of the course of cellulose fermentation at 37 and 65° C. It need only be added that the results obtained from these further experiments were in entire harmony with the conclusions already described in this section of the paper, particularly in regard to the higher degree of stability and consequently greater accumulation of lactic acid when the fermentation is brought about at 65° C. by the thermophilic micro-organisms.

The results of these final experiments demonstrate that the rumen normally contains both types of cellulose-splitting bacteria. Only the micro-organisms capable of fermenting at 37° C., however, are able to bring about those changes in the rumen whereby cellulose is rendered available to the organism. The thermophilic bacteria, which presumably gain entry with the food, are unable to grow and multiply at the temperature of the rumen and are present in relatively small numbers. This is shown by the much longer time required at 65° C. as compared with 37° C. for the attainment of the "head" stage of fermentation when making cultures by inoculation from a common stock of rumen material.

Thus, in the present instance, the "head" stage was reached in 2 days when the incubation was carried out at 37° C., whilst at 65° C. the fermentation only came to the "head" stage on the 6th day of incubation.

This difference by no means warrants the assumption that the thermophilic bacteria are less efficient fermenters of cellulose than the low-temperature micro-organisms. Indeed, if *active subcultures* of the two kinds of cellulose splitters be compared, it is found that the times required for bringing the fermentations to a "head" are approximately the same. When inoculating from the original rumen material, however, the differences in the rates of fermentation are merely a reflexion of the fact that the thermophilic bacteria are present in the rumen in much smaller numbers than the low-temperature micro-organisms.

FERMENTATION OF GLUCOSE BY THERMOPHILIC BACTERIA AT 65° C.

Since such characteristic differences, in respect of pyruvic and lactic acid accumulation, had been noted between the fermentations of cellulose at 37 and 65° C., and since, on the assumption that glucose is one of the primary breakdown products of cellulose during bacterial fermentation, it is postulated that the course of fermentation of glucose and cellulose should display features in common, it was considered probable that similar differences should characterize the fermentations of glucose at the higher and lower temperatures. In order to test this point, 50 c.c. of subculture 5 (made from subculture 4 used in the experiment summarized in Table V) was added to 400 c.c. of the culture medium containing, in addition to the usual salts, 10 g. of separately sterilized glucose. The flask was placed in an incubator at 65° C., and analysis was carried out at intervals during the period of incubation. The results are recorded in Table VII.

Table VII. *Showing course of fermentation of glucose at 65° C.*

| | Amounts per 100 c.c. medium | | | | |
|---|---|---|---|---|---|
| Incubation period days | Glucose g. | Lactic acid g. | Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
| 1 | — | — | — | Faint | No analysis done |
| 2 | 1·75 | 0·29 | 0·10 | ,, | Moderate gas production |
| 3 | 1·45 | 0·67 | 0·11 | ,, | Slight gas production |
| 5 | 1·05 | 0·73 | 0·13 | ,, | ,, |
| 7 | 0·77 | 1·02 | 0·15 | ,, | ,, |
| 9 | 0·54 | 1·37 | 0·19 | Very faint | Rather more gas production |
| 12 | — | 1·57 | 0·38 | Negative | Fairly copious gas production |
| 18 | — | 1·34 | 0·63 | ,, | No gas production |

If the results in Tables I and VII be compared, it will be noted that the fermentation of glucose at 37° C. appears to proceed more readily than at 65° C. The whole of the glucose in the former case had disappeared in 5 days, whereas at 65° C., under the influence of the thermophilic bacteria, about 12 days were required for the complete disappearance of the glucose. The comparison in this respect, however, should be interpreted with reserve, since the subcultures from which inoculation was made may in the two cases have contained widely differing concentrations of the two micro-organisms.

The comparison distinctly confirms the finding that lactic acid constitutes a more stable phase at 65 than at 37° C. In the latter case, the concentration of this acid had fallen to 0·02 % on day 8, whereas at 65° C. the amount was as high as 1·57 % on day 12 and had fallen only to 1·34 % on day 18. The much smaller gas evolution at the higher temperature offers a further indication of the greater resistance to breakdown displayed by the lactic acid under these conditions. Definite reactions for pyruvic acid were obtained in the first stages of the fermentation, but the colour tests were very much weaker than had been obtained in the fermentations conducted at 37° C. Confirmation of these findings was obtained in a duplicate trial at 65° C., the results of which are recorded in Table VIII.

Table VIII. *Fermentation of glucose at 65° C. Duplicate experiment carried out under same conditions as in experiment summarized in Table VII*

| Incubation period days | Amounts per 100 c.c. medium: Glucose g. | Lactic acid g. | Vol. fatty acids as acetic acid g. | Pyruvic acid | Remarks |
|---|---|---|---|---|---|
| 2 | 1·87 | 0·30 | 0·10 | Faint | Slight gas production |
| 4 | 1·18 | 0·69 | 0·13 | Very faint | ,, |
| 7 | 0·55 | 1·10 | 0·13 | ,, | ,, |
| 10 | Trace | 1·59 | 0·17 | ,, | ,, |
| 17 | — | 1·44 | 0·34 | Negative | Very slight gas production |

It may be concluded from Tables VII and VIII that the course of glucose fermentation is affected in a manner similar to that noted with cellulose by employing cultures of thermophilic rumen bacteria at 65° C. instead of cultures of micro-organisms capable of fermenting at 37° C., a finding lending additional weight to the theory that cellulose, during bacterial fermentation, first undergoes hydrolysis to glucose before suffering further breakdown.

It has been shown, therefore, that glucose, pyruvic acid, lactic acid and volatile fatty acids may arise during the breakdown of cellulose by fermentation *in vitro* by rumen bacteria. Of these products, it is unlikely that glucose escapes further breakdown when such bacterial fermentation occurs in the rumen of the animal. To what extent the fat precursors, pyruvic and lactic acids, undergo absorption from the digestive tract, and indeed, in what manner these *in vitro* findings may be applied in attempts to explain the value of cellulose to the ruminant, must be left for discussion until the results of work with sheep with rumen fistulas are published.

## SUMMARY

Further studies of the behaviour of cellulose-splitting bacteria in artificial media have been made in an attempt to account for the manner in which cellulose is utilized for fat production in the ruminant. The behaviour of thermophilic bacteria, with an optimum temperature of fermentation in the neighbourhood of 65° C., has been compared with that of the micro-organisms capable of fermenting at 37° C. In both cases the cultures were obtained from the rumen contents of sheep.

By the addition of toluene at the "head" stage of fermentation, it was possible to demonstrate, both at 37 and 65° C., the production of small amounts of glucose during subsequent incubation. Glucose is clearly a primary breakdown product in the bacterial fermentation of cellulose.

An investigation has been made of the nature of the volatile fatty acids produced by bacterial decomposition of shredded filter paper. No consistent differences from this standpoint were noted between the reactions as carried out at 37 and 65° C., but the nature and proportions of the acids varied considerably from culture to culture. In some cases acetic acid was produced almost exclusively, whilst in others the fatty acids consisted of butyric and formic acids, with but traces of acetic acid. The findings in this regard apparently afford no clue to the manner in which cellulose is used for fat production, since propionic acid, the only recognized fat-former among the lower normal fatty acids, was not detected.

Since glucose is one of the earliest transient phases to arise during the breakdown of cellulose, it was thought that an investigation of the behaviour of this sugar during fermentation by rumen bacteria might shed light on the mechanism of cellulose breakdown. During fermentation of glucose at 37° C. by rumen micro-organisms, pyruvic acid is

formed at an early stage. This gradually disappears from the medium, by reduction to lactic acid and/or by breakdown into volatile fatty acids and gaseous products. With the disappearance of pyruvic acid, lactic acid begins to accumulate in the medium in considerable amount, but the concentration of this acid also declines sharply during the later stages as a consequence of its breakdown to gases and lower fatty acids. Support to these findings was given by separate studies of the breakdown of pyruvic acid and lactic acid by rumen bacteria at 37° C., when these organic acids constitute the sole source of assimilable carbon in the medium.

The fermentation of cellulose at 37° C. takes a course similar to that for glucose, the main difference lying in the much smaller accumulation of lactic acid during the period of incubation. This is explained as being due to the slower rate of production of lactic acid from the more stable cellulose, the amount of this acid in the medium at any given time depending on the difference between the rates of its production from its precursor and its further breakdown into gases and volatile fatty acids.

The course of fermentation of cellulose by thermophilic rumen bacteria at 65° C. was found to be strikingly different, in a quantitative sense, from that associated with the rumen micro-organisms bringing about the fermentation at 37° C. The results were remarkable for the relatively high amounts of lactic acid that were present in the medium during fermentation of the prepared filter paper at 65° C., amounts invariably very greatly in excess of those detected when the fermentation was carried out at 37° C. and, in some cases, of the same order as were found during the fermentation of glucose at 37° C. Lactic acid exhibits a good degree of stability during the fermentation of cellulose at the higher temperature. With pyruvic acid, however, the reverse was true, since only the faintest tests for this acid were obtained at the outset of fermentation at 65° C., whereas strong reactions were noted during the first half of the fermentation at 37° C.

The course of glucose fermentation is similarly modified by employing cultures of thermophilic rumen bacteria at 65° C. instead of rumen bacteria capable of acting at 37° C., a finding which affords additional support to the theory that this hexose is a primary breakdown product of cellulose during bacterial fermentation.

It has been shown, therefore, that glucose, pyruvic acid, lactic acid and volatile fatty acids (formic, acetic and butyric acids) may arise during the breakdown of cellulose by fermentation *in vitro* by rumen bacteria. Of these products it is unlikely that glucose escapes further

breakdown when such bacterial fermentation occurs in the rumen of the animal, and the hypothesis embodying the suggestion that the fat-forming power of cellulose may be attributed to glucose formation in the rumen has been abandoned. To what extent the fat precursors, pyruvic and lactic acids, undergo absorption from the digestive tract, and indeed, in what manner these *in vitro* findings may be applied in attempts to explain the value of cellulose to the ruminant, must be left for discussion until the results of work on sheep with rumen fistulas are published.

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# OVULATION IN THE EWE

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Numerous investigations on the occurrence of ovulation in the ewe have been made by different workers. Apart from its theoretical interest the accurate determination of the time of ovulation is of practical importance in ascertaining the best time for mating. The observations reported in this paper were undertaken to assist in determining the optimum time for the introduction of sperm in artificial insemination experiments.

The earliest record of ovulation in the ewe appears to be that of Bischoff (1844), who observed that ovulation was spontaneous and occurred 24 hr. after the onset of oestrus. Marshall (1903) also concluded that ovulation was spontaneous. Ivanow (1913), cited by Grant (1934), showed that coitus had no effect on the time of ovulation. Ivanow also made numerous observations on the time of ovulation in relation to the period of oestrus. He found in a Russian breed that one ewe out of forty-three killed during the first 24 hr. of oestrus had ovulated, forty-two out of forty-six killed during the second 24 hr. and eight out of nine killed during the third 24 hr. had ovulated. Twenty-six out of twenty-eight ewes killed 1–24 hr. after the end of heat had ovulated; thirty ewes killed on the second day, twenty-eight on the third day and twenty-eight on the fourth day after the end of heat had all ovulated. Ovulation thus occurred during the 24–48 hr. period of heat in the majority of cases, and rarely in the first 24 hr. of heat; also the majority of ewes had ovulated within 24 hr. after the end of heat.

Quinlan & Maré (1931) stated that ovulation rarely takes place before the 36–40th hour of oestrus in the Merino. In one ewe killed at the 40th hour of oestrus the follicle had ruptured a very short time before slaughter, and in another ewe killed at the 41st hour of oestrus the follicle had just ruptured. All ewes killed 12 hr. and more after the end of oestrus had ovulated. The authors concluded that there appears to be little doubt that ovulation occurs towards the end of oestrus. They

also stated that the duration of oestrus appears to depend on the time of rupture of the follicle, and that delay in rupture appears to explain the prolonged oestrus observed occasionally.

Cole & Miller (1932) found that ovulation had not occurred in six Rambouillet ewes killed during the first day of oestrus, but eight out of twelve killed on the second day had ovulated. In a later paper these authors observed that ovulation occurs about the 25th hour of oestrus (1935). Five ewes had not ovulated by tbe 22nd hour, and one ewe had not ovulated by the 30th–32nd hour. They stated that it is agreed by all the more recent investigators that ovulation occurs spontaneously during the second half of the oestrous period, and that judging from their own results it occurs rather uniformly between the 22nd and the 30th hours of oestrus.

Allen *et al.* (1931) did not observe ovulation before the 24th hour after the first appearance of oestrus; unsegmented ova were found in the oviduct between the 24th and 50th hours.

McKenzie *et al.* (1933) found that ovulation had not occurred in seven ewes at 15·5, 19·3, 22, 23, 24·1, 28 and 49·5 hr. respectively after the onset of oestrus, and had occurred in twenty-four ewes observed from 23·7 to 49·5 hr. after oestrus began. Ovulation was actually observed in three instances at 29·8, 34·5 and 37·1 hr.

Clark (1934) found that ovulation occurred rather late in oestrus. Stieve (1934) examined eight ewes immediately before, during and after the end of heat. Ovulation had occurred in three ewes examined after the end of heat. It was concluded that heat persists as long as the ovary contains a mature follicle and ceases when the follicle has ruptured. Grant (1934) killed five ewes during oestrus, at 1, 6, 8, 18 and 24 hr. after the onset of oestrus, and only the ewe killed at 18 hr. had ovulated. It would appear that rupture may occur earlier (at 18–24 hr. after the beginning of heat) in Scottish sheep than in other breeds.

Green & Winters (1935) from an examination of thirteen grade Shropshire ewes found that ovulation occurred late in the heat period as the animal was passing from heat. Ovulation had occurred in five ewes that were off heat when killed and had not occurred in two ewes killed at 8 and 12 hr. after the beginning of heat. Evans & Miller (1935) found a fertilized ovum in the Fallopian tube 24 hr. after oestrus was first noted.

The variation in the time of ovulation noted by different authors may be due to breed and possibly environmental influences, as well as to the accuracy with which the stages of oestrus were timed. It seems,

however, that in the majority of cases ovulation occurs towards the end of heat or shortly afterwards, and that it rarely occurs before 24 hr. after the onset of heat.

## Material and methods

The experimental animals were high-grade Merino ewes and an indigenous breed known as Masai sheep. Vasectomized rams were run with the flock, which was under constant supervision, to pick out ewes on "heat". The time of onset of oestrus was thus known accurately. When a ewe came on heat she was placed in a separate paddock and tested by the "teaser" ram every three hours. Later, tests for heat were made at half-hourly or shorter intervals. It was thus possible to determine with a fair degree of accuracy the stage of oestrus or metoestrus at which the ovaries were examined. When the ovaries of a ewe that was on heat were to be examined the examination was conducted as quickly as possible after the last service to ensure that the ewe would still be on heat at the time of examination.

All ovarian operations were made by laporotomy, in the majority of cases under nembutal anaesthesia. Some ewes were therefore used several times. Prior to rupture, elevation of a small papilla on the surface of the follicle occurs as noted by Grant (1934). When rupture was very recent oozing of blood from the point of dehiscence was invariably noted. When ewes were operated on during oestrus a further examination was made after 2–3 days to discover whether or not ovulation had occurred.

## Results

The results are presented under three headings: examination of ovaries of (1) ewes during oestrus, (2) ewes after the end of oestrus, and (3) ewes with short oestrous periods.

*Examination of ovaries of ewes during oestrus.* Eight laporotomies were performed on six ewes in this series, at intervals ranging from 19½ to 50 hr. after the beginning of oestrus. All the ewes stood well for the ram at the last service, and since the interval between this service and the time of examination of the ovaries was short in most cases it can be concluded that the ewes were actually on heat at the time of examination.

In none of these ewes had ovulation occurred at the time of examination, but all had ovulated when examined 2–3 days later. In five cases the examination was conducted at intervals of from 27½ to 50 hr. after the beginning of oestrus. In contrast, all the ewes examined during this

interval which had been off heat for more than 1 hr. had ovulated (Table II). There was considerable variation in the size of the mature follicle in these ewes which was probably due to the different stages of oestrus at which they were examined. No papilla was observed in any of the follicles. It was therefore concluded that ovulation in the ewe does not occur during oestrus.

Table I. *Examination of ovaries of ewes during oestrus*

| Date | Ewe | No. of hours since onset of heat | State of follicles | Interval between last service and examination of ovaries |
|---|---|---|---|---|
| 6. iv. 36 | 34 | 19½ | No large follicle | 2½ hr. |
| 27. iv. 36 | 33 | 22 | ,, | 15 min. |
| 20. v. 36 | 34 | 24¼ | ,, | 2 hr. |
| 19. v. 37 | M 22 | 27½ | 1 large follicle | 25 min. |
| 30. v. 37 | 220 | 30 | No large follicle | 13 min. |
| 29. v. 37 | M 23 | 37½ | 1 large follicle | 37 min. |
| 9. xii. 36 | 220 | 44 | No large follicle | 15 min. |
| 20. vi. 37 | M 8 | 50¼ | ,, | 10 min. |

*Examination of ovaries after the end of oestrus.* Fifteen laporotomies were performed on twelve ewes at intervals ranging from 30 hr. 45 min. to 73 hr. after the beginning of oestrus. In most cases the operation was performed as soon as possible after the end of oestrus, which was known to within half an hour. The time relationship of ovulation to the beginning and end of oestrus is thus available.

Table II. *Examination of ovaries of ewes after the end of oestrus*

| Date | Ewe | Duration of oestrus hr. | Interval between examination of ovaries and Onset of heat hr. | min. | End of heat hr. | min. | State of follicles |
|---|---|---|---|---|---|---|---|
| 24. vi. 37 | 71 | 30 | 30 | 45 | 0 | 47 | 1 papilla ruptured during examination |
| 16. xi. 36 | 394 | 30¼ | 31 | 25 | 1 | 14 | 1 follicle ruptured |
| 29. iv. 36 | 34 | 26½ | 34 | 0 | 8 | 0 | 2 follicles ruptured |
| 18. xi. 36 | 46 | 33½ | 34 | 0 | 0 | 33 | Small papilla—not ruptured |
| 3. vi. 37 | M 12 | 33½ | 34 | 17 | 0 | 41 | 1 follicle ruptured |
| 30. xii. 36 | M 24 | 34¼ | 35 | 20 | 1 | 10 | ,, |
| 16. xi. 36 | 235 | 35 | 36 | 0 | 1 | 31 | ,, |
| 16. xi. 36 | 379 | 37¾ | 38 | 55 | 1 | 10 | ,, |
| 11. v. 36 | 34 | 38 | 42 | 0 | 4 | 0 | ,, |
| 29. xii. 36 | M 22 | 41¼ | 42 | 17 | 1 | 8 | ,, |
| 11. v. 37 | M 23 | 44¾ | 45 | 45 | 1 | 5 | ,, |
| 16. i. 37 | M 24 | 45½ | 46 | 35 | 1 | 20 | ,, |
| 23. xi. 36 | 436 | 34¼ | 47 | 0 | 13 | 0 | ,, |
| 25. vii. 36 | 436 | — | 72 | 0 | — | | ,, |
| 24. vii. 36 | 511 | — | 73 | 0 | — | | ,, |

In all but two of these ewes the follicle had ruptured. Ovulation occurred as early as 41 min. after the end of heat and in every case had taken place 65 min. or more after the end of oestrus. In the ewes examined shortly after the end of oestrus ovulation had occurred recently.

There is considerable variation in the duration of oestrus in these ewes, and no correlation is evident between the time of ovulation and the length of the interval between onset of oestrus and examination of the ovaries. It appears that ovulation is related to the end rather than to the beginning of oestrus and that it occurs shortly after the cessation of oestrus. Ewe No. 46, for example, had not ovulated when examined 33 min. after the end of heat. There was, however, a small papilla on the follicle, and it is probable that rupture was imminent. During the examination of ewe No. 71, 47 min. after the end of heat, the small papilla on the capsule ruptured. This may, however, have been due to manipulation of the ovary. On the other hand, in ewe No. M 12 the follicle had ruptured when examined 41 min. after the end of heat.

*Examination of ovaries of ewes with short oestrous periods.* The maximum duration of oestrus in this series is 21 hr., which is considerably shorter than the average of the Merino in Kenya (unpublished results). In these ewes it was found that there was no close correlation between the end of oestrus and the time of ovulation as has been noted in ewes with longer oestrous periods. The earliest time at which ovulation was noted was 25 hr. 37 min. after the beginning of oestrus. In this ewe the ovaries were examined 7 hr. 37 min. after the end of heat and ovulation had just occurred.

Table III. *Examination of ovaries of ewes with short oestrous periods*

| Date | Ewe | Duration of oestrus hr. | Interval between examination of ovaries and: Onset of heat hr. | Onset of heat min. | End of heat hr. | End of heat min. | State of follicles |
|---|---|---|---|---|---|---|---|
| 5. vi. 37 | 352 | 11¼ | 19 | 21 | 8 | 0 | 1 large follicle |
| 3. vi. 37 | 370 | 21 | 22 | 25 | 1 | 27 | ,, |
| 19. xi. 36 | 510 | 19 | 23 | 0 | 4 | 0 | Small papilla not ruptured |
| 14. xi. 36 | 39 | 19¼ | 23 | 0 | 4 | 30 | 1 large follicle no papilla |
| 6. vi. 37 | 353 | 18 | 25 | 37 | 7 | 37 | 1 follicle ruptured very recently |
| 21. v. 36 | 35 | 8¼ | 27 | 30 | 19 | 30 | 1 follicle ruptured |
| 2. v. 36 | 35 | 14 | 29 | 0 | 15 | 0 | ,, |
| 13. iv. 36 | 35 | 20 | 29 | 30 | 9 | 30 | ,, |

In three other cases in which the ovaries were examined 27½, 29 and 29½ hr. after the onset of oestrus and 19½, 15 and 9½ hr. respectively after the end of heat, ovulation had occurred. In ewe No. 510, examined 23 hr. after the onset of oestrus and 4 hr. after the end of oestrus, there was a small papilla on the follicle and rupture was probably imminent, but in ewe No. 39, examined at much the same time, there was no sign of imminent rupture. It would thus appear that a minimum period of approximately 23–25 hr. must elapse after the onset of oestrus before ovulation occurs.

## Discussion

There is little change in the size of the follicle during interoestrum. Grant states that apparently during interoestrum one or more follicles grow to a certain size, and at the beginning of oestrus the largest follicles start to grow rapidly. Casida & McKenzie (1932) found that there was a gradual increase in the mean diameter of the follicle during the first third of the cycle, but the follicle was just as large at approximately the mid-point of the cycle as just before ovulation. Grant did not confirm Quinlan & Maré's statement that rapid enlargement of the follicle destined to rupture at the next ovulation occurs shortly after ovulation. The author noted that in the early part of oestrus it was often impossible by naked-eye examination to determine which follicle was going to rupture. Later in oestrus there was usually one follicle which was obviously larger than the others. The appearance of the maturing follicle prior to rupture observed by the author was very similar to that observed by other workers. The capsule of the follicle becomes thinner as ovulation approaches and the follicle acquires a darker appearance. The vascularity of the capsule appears to increase and capillaries in the capsule can usually be seen. Before rupture occurs a small papilla is elevated on the surface of the capsule. The first reference to this papilla appears to have been made by Grant. It was noted by the author in every case in which rupture was about to occur.

According to Quinlan & Maré the point of rupture is indicated by an opening with somewhat rugged borders. The border is stained with coagulated blood and a small blood clot rapidly closes the opening. Cole & Miller (1935) stated that the occurrence of ovulation is readily determined by prominent rupture points protruding from the surface of the ovary. A blood clot appears at the point of rupture. Green & Winters (1935) stated that at the point of rupture a red area denoting the location of the former follicle is observed with little difficulty. In

the author's experience the first sign of rupture is oozing of blood from the papilla on the surface of the follicle. It would appear that ovulation in the ewe is a quiet gradual process, similar to that observed by Walton & Hammond (1928) in the rabbit, and not a sudden ejaculation of follicular contents. This view agrees with Hartman's statement that ovulation is not a cataclysmic process but a gradual opening of the follicle (1932). On the other hand, Hill *et al.* (1935), from a cinematographic study of ovulation in the rabbit, concluded that ovulation is truly explosive in nature. It is conceivable that the oozing of blood from the follicle of the ewe might appear to be more in the nature of an ejaculation if examined under greater magnification such as used by these authors in the rabbit.

Ovulation did not occur in any of the ewes examined by the author while they were still on heat. There are some records of ovulation occurring during heat (Quinlan & Maré, 1931; Cole & Miller, 1932; Grant, 1934), though it is somewhat difficult to decide from the accounts given whether or not the ewes were actually on heat at the time of killing. There is no doubt, however, that in the present experiment when a ewe was examined while still on heat after 30 hr. from the onset of oestrus ovulation had not occurred, while if a ewe had been off heat for more than 1 hr. after this interval ovulation had occurred.

Most workers have found that ovulation had occurred when ewes were examined after the end of heat. There is considerable variation, however, in the time of ovulation noted by different workers, as, for example, the three ewes in which ovulation was actually observed at 29·1, 34·5 and 37·1 hr. after the beginning of heat by McKenzie *et al.* (1933), and the observations of Quinlan & Maré (1931), who found that ovulation occurred from the 36th to the 40th hour of heat. In the present experiment in ewes with oestrous periods of 30 hr. or more, the time of ovulation was related to the end rather than to the beginning of heat, and since the duration of oestrus varies considerably in the ewe it is thought that much of this variation in the time of ovulation must be due to differences in the length of heat.

It is quite clear that in ewes with short heat periods rupture of the follicle occurs some considerable time after the end of heat and that there is a minimum period of approximately 23–25 hr. before which ovulation does not occur, irrespective of the length of heat. There are few records by other workers of ovulation occurring in the first 24 hr. after the beginning of heat.

Quinlan & Maré's statement that the duration of oestrus appears to

depend on the time of rupture of the follicle and that delay in rupture appears to explain the prolonged oestrus occasionally observed is not substantiated. Stieve (1934) also concluded that heat persists as long as the ovary contains a mature follicle and ceases when the follicle ruptures. The ewes with short heat periods examined by the author are in contrast to this view, for oestrus had ceased several hours—in one case 7½ hr.—before ovulation occurred. Moreover, in ewe No. 46, examined 33 min. after the end of heat, the follicle had not ruptured, and in ewe No. 71, 47 min. after the end of heat, ovulation was probably just about to occur. It therefore seems that normally oestrus ceases before rupture of the follicle occurs in the ewe, as in the cow (Hammond, 1927).

## SUMMARY

Thirty-one laporotomies, in addition to a number of confirmatory operations, were performed to determine the time of ovulation in the ewe. It was found that (1) ovulation did not occur during oestrus, (2) in ewes with oestrous periods lasting 30 hr. or more ovulation occurred a short time after the end of oestrus and that the time of its occurrence is related to the end and not to the beginning of oestrus, and (3) there is a minimum period of approximately 23–25 hr. before which ovulation does not occur, irrespective of the duration of oestrus.

[*Postscript*, 7 *November*. Since the above was written a further memoir has been published by McKenzie and Terrill (1937) dealing with oestrus, ovulation and the related phenomena in Hampshire, Shropshire, Southdown and Rambouillet sheep. These authors found that the time of ovulation varied from earlier than 12 hours to later than 41 hours after the onset of oestrus; further, it was found to occur as early as 11 hours before the end of oestrus and as late as 6 hours after the end of oestrus. Generally speaking, ovulation took place near the end of oestrus.

F. H. A. M.]

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# THE NITROGEN CYCLE IN GRASSLAND SOILS: WITH ESPECIAL REFERENCE TO THE ROTHAMSTED PARK GRASS EXPERIMENT

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(With Nine Text-figures)

## Introduction

Soil under permanent grassland differs essentially from arable soil in that the one is continuously occupied by the crop, while the other is disturbed by periods of intense cultivation alternating with periods of cropping. As a consequence there are noteworthy differences in the behaviour of the two types of soil, differences that are shown particularly clearly in the nitrogen cycle. Grassland soils, as Lawes & Gilbert (1) demonstrated, are relatively high in organic matter and nitrogen; arable soils are much poorer in these respects. "When arable land is converted into meadow there is an increase in vegetable matter, carbon and nitrogen, and an accumulation of fertility within the soil.... When, however, grassland is broken up and converted into arable, the accumulated fertility rapidly diminishes" (Lawes (2)).

The bulk of the earlier work on the nitrogen cycle in soils has been restricted to arable land, and it seemed therefore desirable to study more closely the changes under the highly contrasted conditions of grassland. Particular attention was given to those products of the nitrogen cycle which are most readily available as plant nutrients: namely, ammonia and nitrate. The levels of ammonia and nitrate in the soils of various types of grassland, the rate and manner of disappearance of nitrogen added in these forms, and the production of ammonia and nitrate from the soil organic matter on incubation, were examined. The results are considered in relation to such factors as the history of the soil and its herbage, the nitrogen content, reaction, and moisture content of the soil, the extent of worm action, and, of course, the season.

The first measurements of soil ammonia and nitrate content were made

in Stackyard Field, Rothamsted from December 1929 to June 1930 (Richardson (3)). This field had been put down to grass about 18 months previously; when the experiment began, it was being grazed as pasture, and in the spring of 1930 it was mown repeatedly. A low ammonia content (generally about 2 mg. N/kg. dry soil in the fresh soil, sampled to a depth of 20 cm.), and a still lower nitrate content (generally about 1 mg. N/kg.) were found. Nitrogen added in winter or early spring disappeared fairly rapidly, the nitrogen-treated plots then giving the same low levels of ammonia and nitrate nitrogen as the untreated ones.

In October 1930, soil in another field, Great Knott, under pasture grassland of the same age as that of Stackyard Field, was analysed. In this field were several areas of peculiarly green, lush grass which possibly represented places where feed had been dumped for stock the previous winter, with accompanying trampling and dunging; the soil of these areas was analysed separately. The results outside the green areas were comparable with those of Stackyard Field (ammonia 2·1 mg. N/kg., sampled to a depth of 10 cm.; nitrate 0·9 mg. N/kg.). Within the green areas, however, much higher values were found (ammonia 9·6 mg. N/kg.; nitrate 15·4 mg. N/kg.).

In order to examine a wider range of soils nnder controlled conditions, systematic analyses of the soils of selected plots of the Park Grass experiment at Rothamsted were made. Here manurial treatments, repeated on the same plots since 1856, have produced great differences not only in the yield and composition of the herbage, but also in the reaction and appearance of the soil. Different plots in the same field resemble in appearance the natural grasslands of localities far apart with very different soils and climates. The work on the Park Grass plots was continued for 3 years, giving a considerable accumulation of data which are presented here in a condensed form.

In addition to this main investigation, shorter studies were made of two other grassland soils on the Rothamsted farm; one, Hoos Field, was temporary ley less than a year old; the other, Great Field, was pasture which had been put down from arable 59 years before. Thus the complete series includes soils which have been under different types of grassland for widely varying periods: Hoos Field, about 6 months, temporary ley; Stackyard and Great Knott Fields, 1½–2½ years, pasture; Great Field, about 60 years, pasture; Park Grass, several centuries under grass, mown annually for 75 years.

The work is presented in the following sections:

(A) The Park Grass Experiment: Description.

(B) The ammonia and nitrate equilibria of grassland soils: (1) Park Grass, (2) other fields.

(C) The mineralizable nitrogen of grassland soils, and the influence of reaction on nitrification: (1) Park Grass, (2) other fields.

(D) The uptake of nitrogen from grassland soils.

(E) The organic matter and total nitrogen content of grassland soils; worm action.

(F) General discussion.

### A. THE PARK GRASS EXPERIMENT: DESCRIPTION

The history and early results of the Park Grass experiment were set out by Lawes, Gilbert & Masters in a series of memoirs (4), and the later results, so far as concerns the herbage, were described by Brenchley in a monograph (5) and in more recent publications (6). The action of the different treatments on the soil has not been studied so exhaustively, but various aspects were investigated by Lawes & Gilbert (7), Hall *et al.* (8) and Crowther (9). These investigations will be further considered where appropriate to the present work.

From Lawes & Gilbert's (4) account of the land prior to the experiment, it is sufficient to note that: "The experiments were commenced in 1856. They have been conducted on a portion of the Park at Rothamsted, where the land has probably been in grass for some centuries. The land is a somewhat heavy loam, with red clay subsoil, resting upon chalk, and although it is not artificially, it is thus naturally, well drained. Lastly, the area selected is very level, and at the time the experiments were commenced the character of the herbage appeared fairly uniform over the whole of it."

From the point of view of the present investigation the outstanding effect, apart from the differences in yield summarized in Table II, is the suppression of the Leguminosae on plots receiving nitrogen (e.g. treatments 9 and 14) and their abundance on the plots receiving minerals only (e.g. treatment 7).

In early 1929 there was a severe frost and drought, during which the herbage on all the unlimed plots receiving sulphate of ammonia was completely killed (Brenchley (10)). The herbage was allowed to recuperate naturally, by seeding or growth from the edges; although plot 9 (complete artificials) appeared to have filled up again in the summer of 1930, it still showed bare patches of soil when the present work began in New Year 1931.

*Soil.* The top soil is somewhat lighter in texture than that over most

of the farm, being described by the Geological Survey as "Lighter clay-with-flints", and by Linwood Lee (11) as a "Deep brown very mellow loam with some fine sand and very fine sand" (Lee's "Hatching" series). The subsoil is heavier.

The whole profile may be divided into rather ill-defined horizons, the appearance of the soil suggesting a slightly degraded (or podzolized) brown earth.

Analyses kindly made by S. P. Aiyar (12) on clay fractions from samples typical of the different horizons gave the following results:

Table I

| Depth of sample cm. | Ratios in clay fraction | | |
|---|---|---|---|
| | $SiO_2/R_2O_3$ | $SiO_2/Al_2O_3$ | $Fe_2O_3/Al_2O_3$ |
| 0–10 | 2·38 | 3·13 | 0·32 |
| 20–30 | 2·25 | 2·98 | 0·32 |
| 55–65 | 2·44 | 3·13 | 0·28 |
| 90–100 | 2·17 | 2·77 | 0·28 |

These results show that there had been no appreciable washing down of sesquioxides. The higher sesquioxide content of the deepest samples is probably associated with a slight change in the parent material, for there is no increase in the $Fe_2O_3/Al_2O_3$ ratio, such as would be expected from leaching down to this depth. Doubtless the action of worms and plants in bringiug soil and bases to the surface has offset the leaching effect of drainage water.

At the present time there are marked differences between the surface soils of some of the plots, differences that extend to the plot boundaries and then cease abruptly, which can only be the result of the long continued treatments. Most obvious to the eye is the fibrous, peaty mat of partly decomposed vegetation on the highly acid plots that have received sulphate of ammonia without lime. This mat, although broken up, has not been destroyed by the yearly harrowing the plots receive. Associated with it is a shallower depth than usual (10 cm. or less) of the dark surface horizon, and a more friable, crumbly, condition of the soil as a whole.

The soils of other plots show certain less-marked differences, for example in structure and in depth of the dark surface horizon, which varies between about 10 and 20 cm. Plot 13 L (organic manures, limed) is more thoroughly worked by moles than any other on the field, and its soil is correspondingly loose and uneven. The soil of plot 14 U (nitrate of soda and minerals, unlimed) is more tenacious in the borer, and stickier to handle when moist, than that of other plots.

The soil reaction of the various plots differs greatly, giving a range of

values all the way from neutrality to extreme acidity. This in itself lends additional interest to a study of the nitrogen cycle in these soils. *p*H determinations made colorimetrically in the course of the present work have shown only minor variations from those recorded by Crowther (9) 10 years earlier, the chief difference being a further reduction in acidity through subsequent liming (Table II).

*Choice of plots.* Out of the forty-five plots of the Park Grass experiment, ten were selected to form a comparable series which should include the action of nitrogen applied in different forms. They comprised pairs of plots, one limed and one unlimed, receiving (*a*) no manure, (*b*) organic nitrogen only, (*c*) minerals only, (*d*) minerals plus sulphate of ammonia, and (*e*) minerals plus nitrate of soda. The treatments and some of their effects on herbage and soil are given in Table II.

Table II. *Data for selected Park Grass plots*

| Plot no. | Treatment | Mean yield of hay 1st crop cwt. per acre 1911–30 | 1931–33 | Leguminosae % in herbage 1931–33 | Soil reaction, *p*H 1923 | 1931 and 1933 Mean |
|---|---|---|---|---|---|---|
| 3 U | Nil | 11 | 13 | 6 | 5·7 | 5·6 |
| 3 L | | 11 | 14 | 11 | 6·9 | 7·3 |
| 13 U | Dung and fish meal | 40 | 50 | *— | 4·6 | 4·7 |
| 13 L | | 35 | 44 | — | 5·8 | 6·6 |
| 7 U | P, K, Na, Mg | 26 | 29 | 21 | 5·4 | 5·1 |
| 7 L | | 33 | 30 | 22 | 6·7 | 6·7 |
| 9 U | S/A, P, K, Na, Mg | 32 | 67 | 0 | 4·0 | 4·0 |
| 9 L | | 49 | 63 | 0 | 4·5 | 5·0 |
| 14 U | N/S, P, K, Na, Mg | 52 | 58 | 0 | 6·4 | 6·0 |
| 14 L | | 51 | 57 | 2 | 6·7 | 7·2 |

* Not determined in these years: generally nil.

Details of treatments:

"L": 2000 lb. ground lime per acre every 4th year, applied 21 January 1932.

"Dung and fish meal": 14 tons dung alternating with 6 cwt. fish meal per acre; dressings every 2 years in winter. Fish meal applied, 19 February 1931; dung, 31 December 1932.

"P, K, Na, Mg": 3½ cwt. superphosphate, 500 lb. sulphate of potash, 100 lb. sulphate of soda, 100 lb. sulphate of magnesia per acre annually in winter.

"S/A": 412 lb. sulphate of ammonia (86 lb. N) per acre annually in spring.

"N/S": 550 lb. nitrate of soda (86 lb. N) per acre annually, applied in two half dressings in early and late spring.

## B. THE AMMONIA AND NITRATE EQUILIBRIA OF GRASSLAND SOILS

### (1) *Park Grass*

*Sampling and analysis.*

Methods of sampling grassland soils are subject to various defects, either in sampling at an inadequate number of points (as with the 9 in. cube method of Lawes & Gilbert), or in some tendency to systematic

errors. The disturbance of the plots involved in taking sufficient 9 in. cube samples would have been prohibitive in the present work, so sampling tubes were used. A simple cylindrical tube sets up compression of the core, as may be seen when a rubber stopper is bored with a cork-borer, and in addition it is difficult to use when stones are present. For the present work one or other type of semi-cylindrical sampling tool was used, similar in action to the well-known cheese tester. In grassland soils with these implements there is some tendency for the top few cm. of soil to be pulled out of the sampling tube by surface roots, but any cores obviously deficient in this way were discarded.

Salminen's sampler (13) was used for the determinations of ammonia and nitrate in fresh soils in 1931 and 1932. This is essentially a half-cylinder of narrow bore, with one lip projecting so as to scoop in the soil when the borer is rotated, and a pointed end. Ten to twelve cores were necessary to give the 150–200 g. required for analysis.

For the larger samples needed for the incubation experiments in 1932 and 1933, a simple half-cylindrical sampling tube of somewhat U-shaped section, about 2 cm. internal diameter, was used. Each core weighed about 60–70 g. and six cores were taken per sample. In 1933 the ammonia and nitrate in the fresh soil was determined on a subsample of this material. The variation with depth between 0 and 20 cm. of the sample taken by this tool is considered in the following section.

The sampling depth throughout these experiments was 20 cm., the borer being pushed in to a depth of about 25 cm. and the bottom few cm., which might have suffered compression, being discarded. Two independent samples were taken per plot, in 1931 and 1932 entirely at random, and in 1933 from separate halves of the plot. These duplicate samples were analysed separately, so that the difference between them gave a combined measure of sampling and analytical errors. The samples were collected in corked bottles, which were kept in a cool place until extracts could be made. All extractions were completed on the day of sampling. When soils are allowed to air-dry before the ammonia and nitrate contents are determined, marked changes take place, especially an increase in the ammonia, and the amount of change varies from soil to soil. For work of this type it is essential therefore that the analyses be made on the fresh soils.

The samples were broken up and mixed by hand, because of the difficulty of sieving heavy soils when wet, larger roots and flint fragments being discarded. Samples were subsampled by successive halving, 100 g. being taken for the preparation of the extract, and 50 g. for dry

matter determination (2 days in a steam oven, or 1 day in an electric oven at 105°). Soil extracts were made by the modified Carsten Olsen method described by Crowther & Richardson (14), except that in 1933 potassium sulphate and sulphuric acid were used instead of sodium chloride and hydrochloric acid for the extracting solution. It was found that the chloride solution frothed more than the sulphate, with slower distillation and slightly low recoveries as a consequence. Ammonia was determined by distillation from magnesia into $N/50$ acid, and nitrate in the same solution by further distillation after the addition of powdered Devarda's alloy. It is important in this method to use freshly ignited magnesium oxide, as carbon dioxide introduces an error in titrations with brom cresol green, which is not eliminated in the blank determinations.

Samples were taken at 4-weekly intervals in 1931 and 1932; while in 1933, the usual interval was 8 weeks. In 1931 nine plots were sampled, the limed unmanured plot (3 L) not being included; subsequently the ten plots shown in Table II were all sampled.

*Variation of samples with depth.*

This was examined on plots 9 L (S/A + minerals, limed) and 14 L (N/S + minerals, limed) by W. G. W. Warren on 8 March 1933. The semi-cylindrical sampling tool, 2 cm. internal diameter, was used, cores being taken as usual to 20 cm. depth and subdivided into 5 cm. sections which were collected in separate bottles. Sufficient cores were taken to give at least 150 g. per bottle, and duplicate samples were collected for each plot. The results are summarized in Table III.

Table III. *Variation of grassland soil with depth* (*Park Grass, plots* 9 L *and* 14 L)

All results on oven-dry basis

| Depth cm. | % of total sample (0–20 cm.) found at each depth | | Moisture % of dry soil | | Mineral N mg./kg. | | % of total mineral N (0–20 cm.) found at each depth | |
|---|---|---|---|---|---|---|---|---|
| | 9 L | 14 L | 9 L | 14 L | 9 L | 14 L | 9 L | 14 L |
| 0– 5 | 17 | 18 | 65·3 | 54·8 | 9·8 | 10·0 | 29 | 23 |
| 5–10 | 22 | 22 | 44·9 | 46·6 | 5·4 | 8·9 | 21 | 25 |
| 10–15 | 26 | 26 | 36·2 | 40·4 | 5·3 | 8·0 | 24 | 27 |
| 15–20 | 34 | 34 | 31·4 | 33·7 | 4·4 | 5·6 | 26 | 25 |

The first column shows that a relatively small proportion (about one-sixth of the total sample) came from the top 5 cm. and a relatively large proportion (about one-third of the total sample) from the bottom 5 cm. The continuous increase in density with depth was associated with more compact structure and a decrease in the proportion of organic matter.

The observed decrease in water content with depth may likewise be associated with decreasing organic matter content.

Mineral nitrogen (ammonia + nitrates) showed a similar decrease in concentration with depth, evidently from the same cause. To a considerable extent the increase in density with depth offset this so that each of the 5 cm. layers made an almost equal contribution to the total amount of mineral nitrogen present in the soil, sampled to a depth of 20 cm.

*Ammonia nitrogen in fresh soil.*

The results for all plots at each date of sampling are shown in Fig. 1. Those for plots 9 U and 9 L with added sulphate of ammonia are discussed separately in a later section. The annual means for all other plots are summarized in Table IV. For plots other than 9 U and 9 L the standard error of a single sample based on the difference between independent duplicates, was 0·46 mg. N/kg. dry soil in 1931, 0·44 mg./kg. in 1932 and 0·67 mg./kg. in 1933. (In 1933 fewer cores per sample were taken with a larger sampling tool, and the plots were sampled in separate halves. The systematic difference between halves was trifling, and entirely insignificant statistically.)

On two occasions in 1931, and once in 1933, the independent duplicates from a single plot differed from each other by relatively enormous amounts (12–21 times the standard error of the differences between duplicates). It was evident that some abnormal disturbing factor, perhaps contamination or a mistaken reading, had affected one of the duplicates, and as the lower of the two values in these exceptional pairs was near the mean for the plot concerned, the higher value was rejected.

Table IV. *Mean ammonia nitrogen in fresh soil, Park Grass*

mg. N/kg. dry soil

| Plot | Treatment | 1931 13 samplings | 1932 13 samplings | 1933 7 samplings | Mean of all samplings (33 samplings, except 3 L) |
|---|---|---|---|---|---|
| 3 U | Unmanured | 5·42 | 5·41 | 4·26 | 5·17 |
| 3 L | Unmanured, limed | — | 5·07 | 3·81 | 4·63 |
| 13 U | Organic | 4·72 | 5·38 | 4·94 | 5·02 |
| 13 L | Organic, limed | 6·50 | 6·13 | 4·57 | 5·94 |
| 7 U | Minerals | 4·15 | 3·98 | 4·01 | 4·05 |
| 7 L | Minerals, limed | 6·26 | 6·19 | 5·16 | 6·00 |
| 14 U | N/S + minerals | 6·20 | 6·07 | 4·39 | 5·77 |
| 14 L | N/S + minerals, limed | 7·00 | 7·18 | 4·76 | 6·60 |

*Influence of season.* Relevant meteorological data for the 3 years of the experiment are presented in Fig. 2. 1931 and 1932 were rather

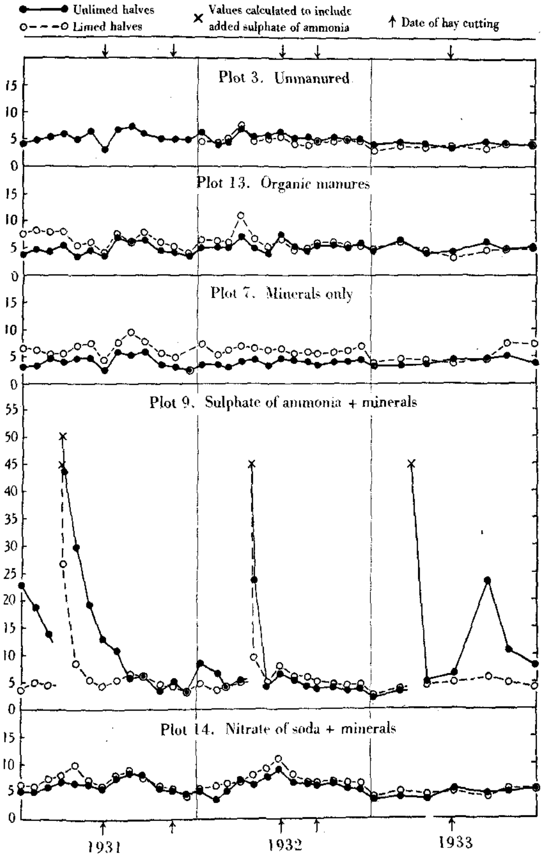


Fig. 1. Ammonia nitrogen in fresh soil (as mg. N/kg. dry soil). Park Grass plots, 1931–3.

similar years, each with a rainfall slightly below the average: 25·68 and 25·94 in. respectively. In 1931 the winter was mild, the summer cool and moist; March was an exceptionally dry month. In 1932 the winter was again relatively mild, while the summer, though marked by hot, sunny

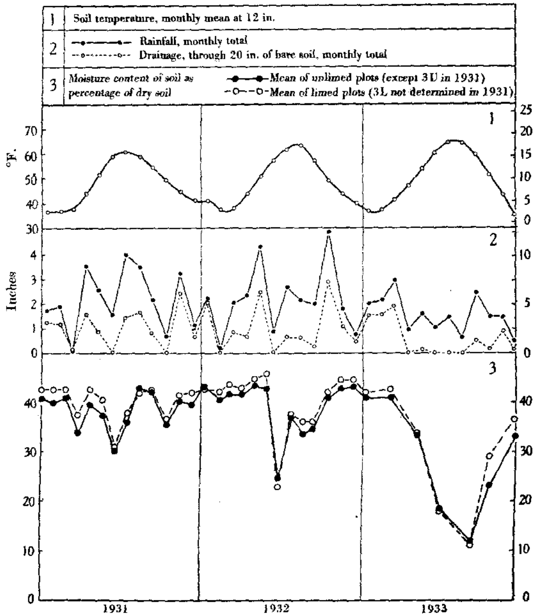


Fig. 2. Meteorological conditions and soil moisture in Park Grass plots, 1931–3.

spells, had adequate rainfall. February was unusually dry, and May and October unusually wet. March was cold, with easterly winds which withered up the pastures. Apart from this, both seasons were favourable for the growth of grass and the yields of the first hay crop and of the aftermath were good.

1933 was a very different year from the other two; the rainfall was exceptionally low (18·62 in.), and leaching through the 20 in. drain gauge (6·83 in.) was about half that of the two previous years. January was a cold month, but most of the year was unusually warm and sunny. The summer was sufficiently hot and dry to check the growth of grass, giving a low yield of hay; by July many of the farm grass fields were brown and parched, and there was not enough growth of aftermath on Park Grass for a second cut to be taken.

The average moisture content of the soil (Fig. 2) showed clearly marked seasonal changes, with a winter level of over 40 % of the dry soil, and summer minima that became progressively lower each year, falling to 11 % in 1933.

There was thus a sufficient range of meteorological conditions to have caused marked changes in the ammonia and nitrate contents of the soil, if these were, in fact, appreciably influenced by seasonal factors.

However, the soil ammonia levels (Fig. 1) showed no clear seasonal changes, but only minor fluctuations. (The plots treated with sulphate of ammonia will be considered separately.) Although many of these were significant in terms of the standard error of a single observation, they cannot be systematically related to changes in climatic factors or the growth of the crop. In any event, the fluctuations were small compared with those in the nitrate content of arable soils or compared with the amount of nitrogen added in the sulphate of ammonia dressings of treatment 9.

The general picture presented by the results is that of a fairly constant ammonia level, subject to small disturbances but on the whole independent of seasonal changes. The average values for the two years 1931 and 1932 (when Salminen's sampler was used) were very similar, the general means for the seven plots that were sampled in both years being 5·75 and 5·76 mg. N/kg. respectively. In 1933 the annual mean of all eight plots was 4·49 as against 5·68 in 1932. The general mean for all plots (excluding 9L and 9U), based on approximately 500 samples, was 5·44 mg. N/kg.

The conditions of the ammonia nitrogen in these soils may be regarded as an equilibrium between the rate of production of ammonia and the rate of its removal. This equilibrium is subject to minor fluctuations, as may be seen in Fig. 1, but the general effect of either season or treatment is relatively small. It was shown above that th mean ammonia content varied by no more than 1·2 mg. N/kg. from year to year; the last column in Table IV shows that under no manurial treatment did the

equilibrium level differ from the general mean by more than 1·4 mg. N/kg.

*Influence of treatment.* In an experiment such as this, laid out with single plots often rather widely separated, it is strictly speaking impossible to distinguish between the effects of treatment and of soil heterogeneity. Here, however, after 80 years of continuous treatment, the plots are themselves fairly uniform but show marked and abrupt changes in both herbage and soil at their boundaries; it seems fair to assume, therefore, that a great part of any difference between the plots is due to the treatment.

The mean soil ammonia contents—the equilibrium levels—under the various treatments show differences which appear highly significant in terms of the standard error of duplicates taken within a plot (Table IV). The annual treatment means for 1931 and 1932 (two similar seasons, the soils being sampled by the same sampling tool) were highly correlated. In these years, the lowest ammonia levels were found on plot 7 U (minerals, unlimed), the highest on plot 14 L (nitrate of soda + minerals, limed); the organic manured plots did not give exceptionally high ammonia contents, nor the unmanured plots exceptionally low ones.

In 1933, however, with a different type of season and another sampling tool, the eight treatment means were not significantly correlated with those of the previous year. The differences between the plots were not, therefore, independent of external influences. As has already been remarked, these differences were in fact quite small.

The most noticeable effect of treatment was that due to liming. This effect can the more confidently be attributed to the treatment because the limed and unlimed plots were adjacent throughout. With treatments 13 L and 13 U (organic manures), 7 L and 7 U (minerals only) and 14 L and 14 U (nitrate of soda + minerals), the ammonia content was higher in the limed than in the unlimed plot, not only in the general means but in eight out of the nine separate comparisons given by the annual means. It is clear, indeed, in Fig. 1 that at most dates of sampling the limed plots were above the unlimed plots. There was a temporary reversal of the effect during the drought of 1933, when (as will be shown later) greater damage appeared to be caused on the more acid plots; this allowed a little ammonia to accumulate but the amount was only of the order of 1 mg. N/kg.

The unmanured plots (3 L and 3 U) differed from the others in giving a slightly lower ammonia content with liming than without, but the difference between limed and unlimed plots was less than with the other treatments.

It is difficult to find an explanation that will cover all of these facts.

It will be shown later that nitrification in the laboratory was more active on the limed than on the unlimed plots: this would have been expected to give lower, rather than higher, ammonia levels with liming. Some of the limed plots are richer in Leguminosae than the unlimed, but this does not explain why 3 L (where Leguminosae are present) should be lower than 3 U, nor why 13 L and 14 L (where Leguminosae are practically absent) should be higher than 13 U and 14 U. It can only be suggested in general terms that liming, by encouraging microbiological and especially bacterial activity, caused a greater "ammonia pressure", and so a rise in the ammonia equilibrium level on the limed plots. The small negative effect on the unmanured plots may be due to some limiting factor, such as phosphate deficiency, which has been accentuated by liming.

The application of lime (on 21 January 1932) did not itself have any effect on the soil ammonia content.

The application of organic forms of nitrogen to plots 13 U and 13 L (fish meal on 19 February 1931 and farmyard manure on 31 December 1932) caused no increase in the soil ammonia: as rapidly as any ammonia was liberated from these manures it was removed by micro-organisms or herbage.

*Sulphate of ammonia.*

The plots (9 L and 9 U) receiving sulphate of ammonia have so far been excluded from the discussion. Wheu they were free from the influence of a recent nitrogen application or of abnormal weather conditions, their behaviour was similar to that of the other plots, with an equilibrium ammonia content around 5 mg. N/kg., and with the limed plot slightly above the unlimed (see Fig. 1). The limed plot showed no disturbance from frost or drought; added ammonia (applied at the rate of about 40 mg. N/kg.) disappeared in a few weeks. The unlimed plot differed from all the others in showing marked effects of climatic factors. As was mentioned in the introduction, the herbage was killed by frost and drought in 1929; it was left to recolonize itself, and there was still much bare soil between scattered tufts of *Holcus lanatus* when the sampling began. Ammonia had accumulated in the soil in the absence of vegetation; there were 23 mg. N/kg. at the beginning of 1931, which decreased slowly to about 10 mg. N/kg. at the time of application of sulphate of ammonia. The next determination, 44 mg. N/kg., incidentally supplied a useful check on the method of sampling and analysis: the value was not far from the calculated 10 + 40 mg. N/kg.

The disappearance of the added nitrogen was slow, evidently because

of the scattered growth and slow nitrogen uptake of the herbage. The nitrogen was not completely removed until mid-August. During the following winter there was a slight, temporary rise in ammonia content. By the spring of 1932, although the *Holcus* was still tufty, the soil between was well covered with seedlings, and in this year the added sulphate of ammonia disappeared almost as rapidly on the unlimed as on the limed plot. In the spring of 1933 the same was true. Later, the hot, dry summer led to "burning" of the herbage on all plots; on plot 9U the individual tufts of *Holcus* looked fairly green, but there was much "dead" soil between them. This allowed ammonia to accumulate, 23 mg. N/kg. being found on 9 September just before the drought broke; subsequently until the end of the year plot 9U remained rather higher than 9L.

The occurrence of fluctuations of the order of those observed in plot 9U emphasizes the relative steadiness of the ammonia equilibrium levels in the other plots. They occurred in the soil of plot 9U only because it was so acid that much of the herbage was killed by severe frost or drought.

*Nitrate nitrogen in fresh soil.*

The results for all plots at each date of sampling are shown in Fig. 3; they are summarized in Table V. (Plots 14L and 14U, receiving nitrate of soda, are omitted from this table and from the calculation of standard errors). The standard error of a single sample, based on the differences between independent duplicates, was 0·54 mg. N/kg. dry soil in 1931, 0·33 mg./kg. in 1932, and 0·21 mg./kg. in 1933. Two abnormally high determinations were rejected in 1931.

The general level of the nitrate nitrogen was low; on no occasion, except immediately after an application of nitrate of soda, did any plot contain more nitrate than ammonia nitrogen.

Table V. *Mean nitrate nitrogen in fresh soil, Park Grass*

mg. N/kg. dry soil

| Plot | Treatment | 1931 13 samplings | 1932 13 samplings | 1933 7 samplings | Mean of all samplings (33 samplings, except 3L) |
|---|---|---|---|---|---|
| 3U | Unmanured | 0·97 | 0·82 | 1·22 | 0·96 |
| 3L | Unmanured, limed | — | 0·48 | 1·02 | 0·67 |
| 13U | Organic | 2·20 | 1·34 | 1·96 | 1·81 |
| 13L | Organic, limed | 1·68 | 0·93 | 1·71 | 1·39 |
| 7U | Minerals | 0·87 | 0·55 | 1·18 | 0·81 |
| 7L | Minerals, limed | 0·96 | 0·64 | 1·08 | 0·86 |
| 9U | S/A + minerals | 1·40 | 1·07 | 1·25 | 1·24 |
| 9L | S/A + minerals, limed | 1·29 | 1·07 | 1·24 | 1·19 |

*Influence of season.* The annual means for the seven plots sampled in both 1931 and 1932 were 1·34 and 0·92 mg. N/kg.; and for the eight plots

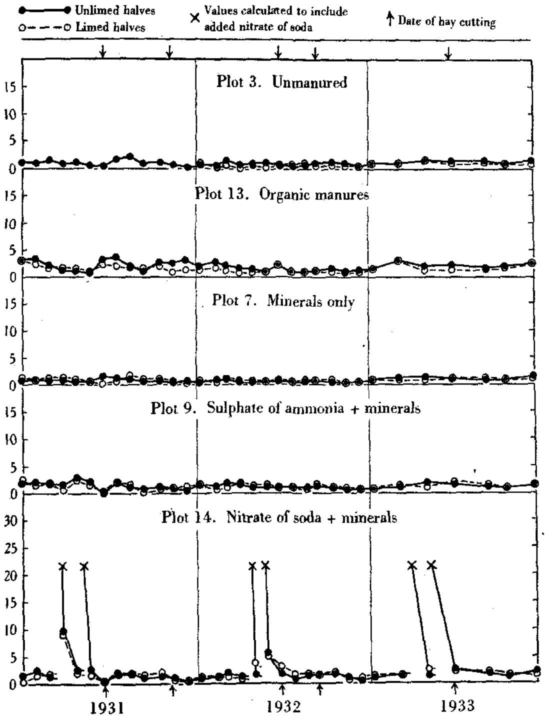


Fig. 3. Nitrate nitrogen in fresh soil (as mg. N/kg. dry soil). Park Grass plots, 1931–3.

sampled in 1932 and 1933, 0·86 and 1·33 mg. N/kg., respectively. The nitrate level was thus lower in 1932 than in either of the other years, but the differences were small. There was no regular seasonal change in

nitrate levels, nor any relation with hay cutting or soil moisture content. There was no accumulation of soil nitrate during the drought of 1933.

The general result resembled that for ammonia, in suggesting an equilibrium level with only minor fluctuations: a balance between the production of nitrate by nitrification and its removal by plants or micro-organisms. The nitrate equilibrium level was below the ammonia equilibrium level, the general mean of some 500 samples (excluding plots 14 L and 14 U) being 1·14 mg. N/kg. The corresponding value for ammonia was 5·44 mg. N/kg.

*Influence of treatment.* The differences between treatments were small, but consistent. The annual treatment means for successive years were significantly correlated. The most marked effect of treatment was that of the organic manures (13 L and 13 U), which consistently gave the highest nitrate level. The unmanured and the "minerals only" plots (3 L and 3 U and 7 L and 7 U) were low throughout, with sulphate of ammonia + minerals (9 L and 9 U) intermediate. There was no appreciable production of nitrate on the latter plots after sulphate of ammonia application.

Liming had a systematic effect, as with the soil ammonia values, but in the opposite direction. In all the pairs of general means, except 7 L and 7 U, and in most of the corresponding annual means, the unlimed plot contained more nitrate than the limed. As with ammonia the relationship is the opposite of that which would be expected from the laboratory nitrification experiments. It may be suggested that liming facilitated nitrate uptake, through the increased supply of calcium ions, so that the equilibrium level was lowered.

The nitrate of soda plots (14 L and 14 U), so far omitted from the discussion, showed no unexpected features. The nitrate, added in two separate half dressings in the spring, always disappeared rapidly, and subsequently the equilibrium levels were similar to those of the other plots. They tended to be above plot 7, but lower than plot 13.

### (2) *Ammonia and nitrate equilibria in other grassland soils*

Ammonia and nitrate determinations were made in other grassland soils over shorter periods than in the Park Grass experiment. The results are brought together here for comparison.

(*a*) *Hoos Field*—temporary ley. Old arable land was sown to Western Wolths ryegrass after barley, in September 1935. The soil was sampled during the spring and summer of 1936 (Norman & Richardson (15)) four independent samples being taken at weekly intervals with the larger,

half-cylindrical sampling tool. There were six cores per sample, the depth being 20 cm. Sulphate of ammonia was applied in the spring; after the added nitrogen had disappeared from the soil a series of practically constant values was obtained, and these agreed with the ammonia and nitrate content of the soil before the nitrogen was added. The mean values, excluding those influenced by the sulphate of ammonia dressing, may be taken as representing the equilibria in this soil. They were, for ammonia nitrogen, 1·07 mg. N/kg.; for nitrate nitrogen, 0·53 mg. N/kg.

(*b*) *Stackyard Field*—pasture, about 2 years old. In this experiment, already mentioned in the introduction, fortnightly sampling was continued over a period of 6 months from December 1929. There was sixfold replication, twelve cores per plot being taken to a depth of 20 cm. with Salminen's sampling tool. The ammonia and nitrate levels were almost constant in the no-nitrogen plots, after an initial rather high value attributable to an autumn application of sulphate of ammonia. Equilibria clearly existed here as in the other experiments. The mean content of ammonia was 2·16 mg. N/kg.; of nitrate, 1·05 mg. N/kg.

(*c*) *Great Field*—pasture, 59 years old. This field was arable land until 1874, when it was laid down under permanent grass. An experiment was put down in the spring of 1933 to test the action of various forms of nitrogen, with sixfold replication of six treatments. The soils were sampled only on two dates, but the values for the no-nitrogen plots agreed sufficiently closely for the mean to be regarded as representing the equilibrium on this soil. Ten cores were taken per plot with the larger, half-cylindrical sampling tool; the depth was only 10 cm., because a bed of flint pebbles below this interfered with sampling. The results for the unmanured plots were:

| | 31 May | 14 June | S.E. | Mean |
|---|---|---|---|---|
| Ammonia, mg. N/kg. | 4·98 | 4·35 | ±0·15 | 4·67 |
| Nitrate, mg. N/kg. | 1·33 | 1·30 | ±0·10 | 1·32 |

The mean value for the mineral nitrogen (6·0 mg./kg.) may be compared with that of the upper 10 cm. of the "depth" samples taken on Park Grass. The mean of 9 L and 14 L, weighted for the relative proportion of soil at each 5 cm. depth (0–5 and 5–10 cm.) was 8·4 mg. N/kg. This refers only to a single date of sampling, but the mean mineral nitrogen from 0–20 cm. in the "depth" samples was not far from the equilibrium value for all plots over 3 years. It appears, therefore, that the mineral nitrogen content of the Great Field soil was rather lower than that of the Park Grass soil.

The results of all the experiments, summarized in Table VI, show a

clear connexion between the ammonia and nitrate equilibria and the length of time the soil had been under grass.

Table VI. *Age and mineral nitrogen equilibria of grassland soils*

| Soil | Years under grass | Depth of sample cm. | Mean ammonia mg. N/kg. | Mean nitrate mg. N/kg. |
|---|---|---|---|---|
| Hoos Field | Less than 1 | 20 | 1·07 | 0·53 |
| Stackyard | 2 | 20 | 2·16 | 1·05 |
| Great Field | 59 | 10 | 4·67 | 1·32 |
| Park Grass | Over 200 | 20 | 5·44 | 1·14 |

Bearing in mind that the shallower sampling on Great Field would tend to give rather high values, it is clear that the "equilibrium" levels of ammonia and nitrate are not constant for all grassland soils, but that they tend to increase with the age of the sward. The rate of change is slow, and falls off with time. A similar slow increase with age is observed in the total nitrogen content of grassland soils (p. 109). It is suggested that as a grassland soil grows older and its organic matter and total nitrogen contents increase, there is a rise in the "ammonia pressure" of the soil. This increase is greater than that in the agencies removing ammonia, with the result that the equilibrium shifts in the direction of a higher ammonia level. With nitrate a similar but smaller effect is observed, most of the increase occurring during the first 2 years.

## C. The mineralizable nitrogen of grassland soils

### (1) *Park Grass*

*Seasonal changes in the nitrogen cycle.*

The approximate constancy throughout the season of ammonia and nitrate levels in a grassland soil does not indicate that climatic factors are without influence on the nitrogen cycle. It shows that when conditions, such as rising temperature, tend to accelerate the "down-grade" side of the cycle, they accelerate also the "up grade" side. The correspondence is not perfect, as is indicated by the small fluctuations in the equilibrium levels, but only on plot 9U of Park Grass were there clear indications of seasonal effects and then only after severe damage to the herbage. Other measures than the determination of field ammonia and nitrate are required to study the influence of season on the soil nitrogen.

Since there is usually no accumulation of surplus mineral nitrogen in the field, it follows that nitrogen made available by the decomposition of the soil organic matter is at once taken up by the plants or by micro-organisms, and the nitrogen found in the grass should be a partial measure

of the activity of the "down-grade" side of the cycle. From various data (including those of Richardson (3)) it can be shown that the amount of nitrogen translocated to the herbage of grassland throughout the year varies in a general way with the soil temperature, from small quantities in the winter to very large ones in early summer. Doubtless the activity of the "down-grade" side, and indeed of the whole, of the nitrogen cycle similarly varies in the field with the soil temperature. Other factors, such as moisture supply will, of course, have some effect.

Another method of studying seasonal changes in the soil nitrogen is to take soil samples at intervals throughout the year, and incubate them under standard conditions. This may be regarded as a way of isolating and accelerating the "down-grade" side of the cycle, and variations in the production of ammonia and nitrate on incubation should correspond with changes in the amount of readily decomposable organic matter in the soil.

The Park Grass soils were examined in this way in 1932 and 1933. It was thought advisable to interfere with the soils as little as possible; preliminary air-drying and the addition of nutrient salts or ammonium compounds, which are adopted in some "nitrification" studies, were avoided in the present work. The fresh soil was transferred to flasks in the incubator on the day of sampling, and there maintained, with occasional shaking, at constant temperature and moisture content for 3 weeks.

*Conditions of sampling and incubation.*

The larger, half-cylindrical sampling tool was used, six cores being taken per sample to a depth of 20 cm. The usual interval between sampling dates was 8 weeks. In 1932 one sample was taken per plot, and in 1933 two samples from separate halves. (It had been observed that fallen leaves were more abundant on one-half of certain plots, and it was desired to test whether this influenced the results, for example by supplying carbohydrates or bases.) In the laboratory, after mixing and subsampling the soil on a plate-glass sheet, duplicate subsamples of 100 g. were put into 350 ml. conical flasks, which were plugged with cotton-wool, weighed, and placed in the incubator at 30° C.

The choice of a standard moisture content was limited by the consideration that the fresh soils could be moistened but not dried. The standard value must be that of the wettest soil likely to be encountered. Fortunately the Park Grass soils are well drained in the field, and are well supplied with organic matter; consequently the mois ure contents employed were not so high as to spoil the condition of the soil in the flasks. Different plots varied in the amount of moisture they held in the field,

so each plot was assigned its own standard moisture content. In 1932 the value chosen was that of the January sample; in 1933 the mean of the five wettest monthly samples of 1932 was taken as giving a more reliable datum line. Actually the 1933 standard values were not very different from those of 1932.

It was necessary to place the flasks in the incubator at their field moisture content; on the following day, when the moisture content of each soil had been determined, the flasks were adjusted to the standard values by the addition of the calculated quantity of distilled water. They were weighed, and made up to this weight with further distilled water at weekly intervals. They were shaken by rotation at intervals of 2 or 3 days. On the 21st day the soils were removed and extracts made for ammonia and nitrate determination. In May 1932, the incubator unfortunately overheated and excessively high values were obtained; the results for this set of determinations have therefore been omitted.

The agreement between duplicate flasks was good in view of the wide variations in nitrifying ability encountered in the different plots. Some of the soils, as will be shown later (p. 93), produced more ammonia and others more nitrate on incubation. The difference between duplicates was naturally less where the ammonia or the nitrate values were low, and to allow for this two sets of standard errors have been calculated, one for the five soils that gave low and the other for the five soils that gave high nitrate values.

*Ammonia and nitrate in incubated Park Grass soils.*

The results for all plots and dates of sampling are shown in Fig. 4; each point represents the mean of two flasks (duplicate subsamples) in 1932, and of four flasks (duplicate subsamples from independent half-plots) in 1933. In spite of their complexity the results present certain well-marked regularities. For example, in some plots ammonia, and in others nitrate, predominated after incubation, and most treatments were consistent in their behaviour throughout the experiment. Plots 13 L (organic, limed), 7 L (minerals, limed), 14 U and 14 L (N/S + minerals, unlimed and limed) produced more nitrate than ammonia at every sampling. On the other hand, plots 3 U (unmanured, unlimed), 7 U (minerals, unlimed), 9 U and 9 L (S/A + minerals, unlimed and limed) gave more ammonia than nitrate at every sampling, with a single exception in 9 L at the end of 1932, when the excess of nitrate was negligible. Plot 3 L (unmanured, limed) gave more nitrate than ammonia except with the first two samplings. As lime was applied to the limed

plots at the beginning of 1932, after a 4-year interval, it is possible that the nitrifying power of 3L had fallen off since the previous liming, and a few months were required for the effect of the fresh liming to penetrate the soil. The only plot which was irregular throughout in its behaviour was 13U (organic, unlimed); here, although on the average ammonia predominated, more nitrate than ammonia was produced with a number of samplings.

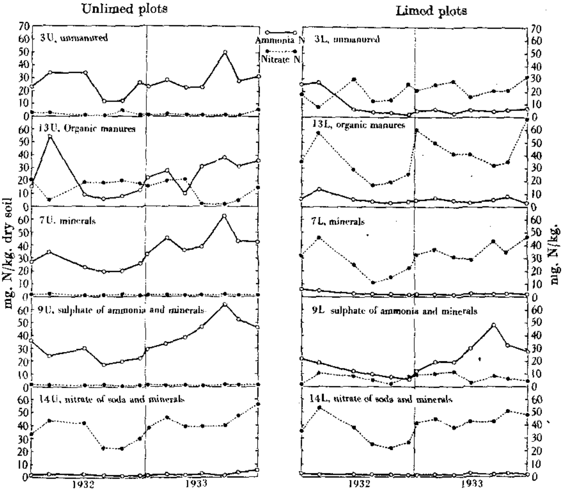


Fig. 4. Mineral nitrogen in incubated soils (as mg. N/kg. dry soil), Park Grass plots, 1932–3.

The nitrifying power of the different soils, as shown by these results, is discussed in a later section (p. 100). It may be pointed out here that the soils in which ammonia predominated were all acid, with a $p$H below 6·0.

The sampling in separate half-plots adopted in 1933 supplied confirmation, if that were necessary, that the differences between plots were actually due to treatment. When the adjacent limed and unlimed plots differed in their relative ammonia and nitrate production, it was found that the two halves of one plot resembled each other, whereas the two adjoining halves of adjacent plots were sharply different.

The influence of season and treatment on the condition of the soil nitrogen can best be discussed in terms of the net production of mineral nitrogen on incubation, obtained by subtracting the mineral nitrogen present in the fresh soil from that found after incubation. The values so obtained are called the *mineralizable nitrogen; ammonifiable* and *nitrifiable* nitrogen may be calculated similarly. "Ammonifiable nitrogen" was sometimes negative, when a soil contained less ammonia after incubation than before.

*Since, except on plot 9U, there were no marked fluctuations in the* mineral nitrogen content of the fresh soil, the values for mineralizable nitrogen resemble, at a slightly lower level, those obtained by adding together the total ammonia and nitrate nitrogen shown in Fig. 4. They are therefore not presented separately in detail.

*Influence of season on mineralizable nitrogen.*

Most of the curves in Fig. 4 show signs of seasonal effects, which are brought out more clearly in Fig. 5 by taking the mean mineralizable nitrogen at each sampling date for two groups of plots. In one group are

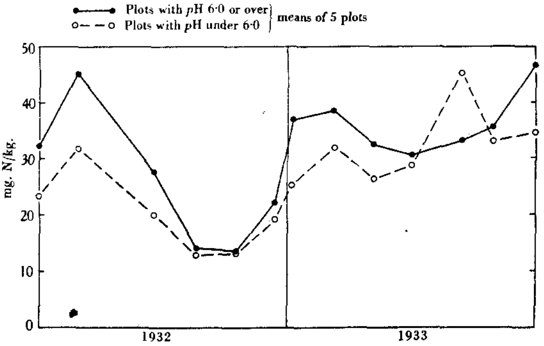


Fig. 5. Mineralizable nitrogen (as mg. N/kg. dry soil) in Park Grass soils, 1932–3.

the five plots with a *p*H of 6·0 or over, which produced chiefly nitrate on incubation, and in the other group are the five strongly acid plots which produced predominantly ammonia. In general the two sets behaved similarly, with the more acid group rather below the other, but at the

climax of the drought of 1933 (6 September) the more acid group showed a sudden peak in mineralizable nitrogen.

The general tendency was for a rise in mineralizable nitrogen to occur during late autumn and winter followed by a fall in late spring and summer. This is the converse of the seasonal temperature rhythm. It is readily interpreted in terms of changes in the soil organic matter. During late autumn and winter a proportion of the grass roots and herbage dies off, leaves being carried into the soil by worms; this increases the amount of readily decomposable organic matter, and hence of mineralizable nitrogen, in the soil. During the spring, as the soil warms up, the organic matter decomposes and the liberated mineral nitrogen is taken up by the herbage. As a result, mineralizable nitrogen levels are low until a fresh supply of organic matter is added in the following autumn.

Any event which damages the herbage severely enough to add further readily decomposable organic matter to the soil will cause a rise in the mineralizable nitrogen at other times of the year. This explains the peak that occurred with the more acid plots in the drought of 1933. On the most acid soil of all, plot 9U, the herbage was so badly damaged that ammonia nitrogen accumulated in the field; on the others, although the damage was less severe, it was sufficient to cause a marked rise in the mineralizable nitrogen on incubation. Even the less acid plots showed a slow rise during the drought, instead of the late summer minimum observed in 1932, and their December level was much higher than in 1932. This is reflected in the general mean of all samplings and all treatments, which was 23·1 mg. N/kg. in 1932 but 34·2 mg. N/kg. in 1933.

*Influence of treatment on mineralizable nitrogen.*

The treatment means for ammonifiable and nitrifiable nitrogen are given in Table VII (six sampling dates in 1932 and seven in 1933).

Table VII. *Ammonifiable and nitrifiable nitrogen in Park Grass soils*

mg. N/kg. dry soil

| Plot | Treatment | Ammonifiable 1932 | Ammonifiable 1933 | Ammonifiable Mean | Nitrifiable 1932 | Nitrifiable 1933 | Nitrifiable Mean |
|---|---|---|---|---|---|---|---|
| 3U | Unmanured, unlimed | 18·0 | 24·4 | 21·2 | 1·7 | 1·0 | 1·3 |
| 3L | Unmanured, limed | 6·6 | 1·5 | 4·1 | 17·5 | 22·5 | 20·0 |
| 13U | Organic, unlimed | 11·6 | 23·2 | 17·4 | 15·4 | 9·6 | 12·5 |
| 13L | Organic, limed | 0·2 | 0·5 | 0·3 | 29·7 | 45·5 | 37·6 |
| 7U | Minerals, unlimed | 21·3 | 39·0 | 30·1 | 1·0 | – 0·1 | 0·5 |
| 7L | Minerals, limed | – 3·2 | – 3·2 | – 3·2 | 24·4 | 35·2 | 29·8 |
| 9U | S/A + minerals, unlimed | 20·0 | 35·7 | 27·8 | 0·3 | – 0·4 | 0·0 |
| 9L | S/A + minerals, limed | 7·0 | 22·2 | 14·6 | 5·0 | 6·1 | 5·6 |
| 14U | N/S + minerals, unlimed | – 4·0 | – 1·9 | – 3·0 | 31·4 | 42·1 | 36·7 |
| 14L | N/S + minerals, limed | – 4·9 | – 3·0 | – 3·9 | 31·9 | 42·4 | 37·2 |

It is evident that in ammonia or nitrate production most of the plots were consistent from year to year. Further, those plots with high nitrifiable nitrogen which gave negative values for ammonifiable nitrogen did so in both years.

In mineralizable nitrogen (Table VIII), the effects of treatment in the 2 years were less consistent. This is attributable to the great difference in the summers of the 2 years, for, as has been shown, the drought of 1933 affected the more acid plots more severely than the less acid plots, and this raised the annual means of the former group disproportionately. It is legitimate, in looking for a general comparison, to exclude the sampling date (6 September) on which the sudden peak occurred in the more acid plots; when this is done the treatment means of the remaining six sampling dates of 1933 compare satisfactorily with the treatment means for 1932, the correlation being now just over the 0·02 level of significance ($r = +0{\cdot}72$).

Table VIII. *Mineralizable nitrogen in Park Grass soils*

mg./kg. dry soil

| Plot | 1932 | 1933 All samplings | 1933 Excluding 6 Sept. | Mean All samplings | Mean Excluding 6 Sept. |
|---|---|---|---|---|---|
| 3U | 19·6 | 25·4 | (22·0) | 22·5 | (20·8) |
| 3L | 24·2 | 24·0 | (24·5) | 24·1 | (24·4) |
| 13U | 27·0 | 32·8 | (32·8) | 29·9 | (29·9) |
| 13L | 29·8 | 46·0 | (48·2) | 37·9 | (39·0) |
| 7U | 22·3 | 39·0 | (35·7) | 30·6 | (29·0) |
| 7L | 21·2 | 31·9 | (30·7) | 26·6 | (26·0) |
| 9U | 20·3 | 35·3 | (34·4) | 27·8 | (27·4) |
| 9L | 12·0 | 28·3 | (25·1) | 20·2 | (18·6) |
| 14U | 27·3 | 40·2 | (41·1) | 33·8 | (34·2) |
| 14L | 27·0 | 39·4 | (39·5) | 33·2 | (33·2) |

Since the normal treatment means (excluding the "abnormal" drought samples) showed consistent effects in the 2 years they are further examined below.

The unmanured plots, limed and unlimed, gave roughly similar amounts of mineralizable nitrogen in both years. They were thus less affected by season than the other treatments, which gave considerably more mineralizable nitrogen in 1933 than in 1932.

The highest values in both seasons were from the limed organic manure plot (13L). Although the unlimed organic plot (13U) was considerably lower than the limed plot in 1933, both of the organic plots were well above the corresponding unmanured plots in the 2 years. Thus the mineralizable nitrogen values pick out at least the broadest distinctions

between soils that might be expected to have high or low available nitrogen (see also p. 98).

Turning to those treatments receiving artificial fertilizers, the plots treated with minerals only (7 U and 7 L) supply the "basal" manuring for comparison with those receiving both nitrogen and minerals. It was found in both years that sulphate of ammonia (9 U and 9 L) lowered the mineralizable nitrogen and nitrate of soda (14 U and 14 L) increased it, comparing either limed or unlimed plots. The benefit from nitrate of soda may be attributed to the heavy yields of nitrogen-rich herbage that it produced, leaving larger residues of readily decomposable nitrogen in the soil. The depression with sulphate of ammonia is less easy to understand, unless the great acidity of both limed and unlimed plots somewhat retarded decomposition.

Liming had a variable effect. It would be expected to cause more rapid decomposition of organic matter and hence larger mineralizable nitrogen values. This was observed with the unmanured plots and those manured with organic matter, where the limed plots were higher than the unlimed in both years. But on those plots receiving minerals, with or without added nitrogen, there was no response to liming. Indeed, there was a tendency, most clearly marked with sulphate of ammonia, for mineralizable nitrogen to be lower on the limed than on the unlimed plots.

This result on plots 9 L and 9 U is a striking one, for it has been said in the past that the "mat" of partly decomposed herbage which accumulated on plot 9 U, as well as on the other unlimed sulphate of ammonia plots, was formed because the intense acidity of the soil retarded the decay of the organic matter. Evidently this is only a part of the explanation, since when soil and organic matter are mixed they decompose rapidly enough to produce more mineralizable nitrogen in the unlimed than in the limed soil, and indeed an amount comparable with that on many of the other plots. Probably the primary cause of the formation of a "mat" on these plots is the complete absence of worms, which appear to have been repelled by the very acid soil conditions. In consequence, the dead herbage does not enter the soil: once the mat is mixed with the soil it readily decomposes.

The result shows the importance in reclaiming matted grassland of breaking the mat up and mixing it with the soil, along with a dressing of mixed fertilizer to assist the cellulosic material to rot down. Even without liming matted material should decompose when this is done.

Because of the variable effect of lime with the different treatments, the mineralizable nitrogen values do not show a general relationship with soil reaction.

*The relation between mineralizable and available nitrogen.*

The nitrogen present in the non-leguminous portion of the herbage is here taken as a measure of "available" soil nitrogen: this is the amount of nitrogen which has actually been taken up from the soil by the plants. The figures (Table IX) for the total herbage are based on nitrogen determinations kindly made by R. G. Warren; separate analyses of the non-leguminous herbage were not made, but from the botanical analysis (6) and a figure for the percentage of nitrogen in the leguminous fraction

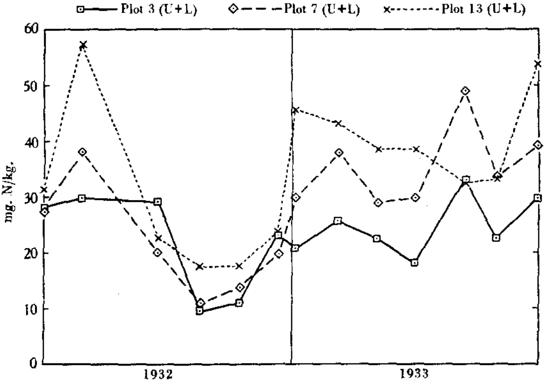


Fig. 6. Mineralizable nitrogen (as mg. N/kg. dry soil) in Park Grass soils, 1932–3. Plot 3, unmanured; plot 7, minerals only; plot 13, organic manures.

based on a number of analyses of legume hay, an estimated correction has been made for nitrogen due to Leguminosae. The result, while approximate, is undoubtedly of the correct order.

The treatments examined are those which have not received added nitrogenous fertilizers: namely, unmanured, organic manures, and minerals only. The production of mineralizable nitrogen by the incubated soils throughout the 2 years 1932 and 1933 is shown in Fig. 6, as the means of limed and unlimed plots. The relative position of the treatments was fairly regular, the unmanured plots being below the organic manured plots on most dates of sampling, with the mineral manured plots intermediate. The means for the "normal" values for the 2 years (i.e. excluding 6 September when the drought affected some plots more than

others) are given in Table IX along with the mean amount of nitrogen in the herbage for the main crops of 1932 and 1933.

Table IX. *Available nitrogen (in herbage) and mineralizable nitrogen, Park Grass,* 1932–3

| | | Mean nitrogen in herbage (main crops) | | Mean mineralizable nitrogen in soil | |
|---|---|---|---|---|---|
| Plot | Treatment | Total herbage lb./acre | Excluding Leguminosae lb./acre | mg./kg. | lb./acre |
| 3 U and 3 L | Unmanured | 19·7 | 17·9 | 22·6 | 49 |
| 13 U and 13 L | Organic | 52·4 | 52·4 | 35·3 | 76 |
| 7 U and 7 L | Minerals only | 40·4 | 26·4 | 27·4 | 59 |

The values for mineralizable nitrogen converted to lb./acre for a depth of 20 cm. run almost parallel with the nitrogen contents of the non-leguminous herbage, exceeding these quantities by about 30 lb./acre. This may be no more than an interesting coincidence, but it none the less suggests that the mineralizable nitrogen is a rough index to the nitrogen available to the plant. However, as Fig. 6 shows, determinations on individual sampling dates cannot be relied on to place the treatments in the correct order. Liming also introduces a disturbing factor, for the separate limed and unlimed plots show a less regular relationship than their means.

Other work in this Department has shown a relationship to exist between nitrogen response in fertilizer field experiments and mineral nitrogen in incubated soils. These soils were first air-dried, and it is possible that air-drying, which greatly increases the values on incubation, eliminates some of the seasonal fluctuations observed with the fresh soils.

### (2) *Mineralizable nitrogen in other grassland soils*

Determinations of mineralizable nitrogen were made on the soils of three fields grazed under ordinary farming conditions, on single sampling dates. In addition, mineralizable nitrogen and $CO_2$ production were measured at weekly intervals in soil samples from the Hoos Field temporary ley experiment (p. 88). The conditions of sampling (depth 0–20 cm.) and of incubation were similar to those for Park Grass soils. $CO_2$ production was determined by Orchard's method (16), small tubes of KOH solution being placed in the stoppered flasks, which were aerated at 2–3-day intervals; the $CO_2$ absorbed by the KOH was measured by titration at the end of the incubation period.

The results for the three grazed fields appear in Table X.

Table X. *Mineralizable nitrogen in grassland soils*

mg. N/kg. dry soil

| Field | Sampling date | Reaction pH | Fresh soil Amm. | Fresh soil Nit. | Incubated soil Amm. | Incubated soil Nit. | Incubated soil Mineralizable |
|---|---|---|---|---|---|---|---|
| *High Field (old park)* | 25. viii. 32 | 5·1 | 3·3 | 1·7 | 13·8 | 14·5 | 23·3 |
| Stackyard (young pasture) | 25. viii. 32 | 6·5 | 1·9 | 0·8 | 1·2 | 25·8 | 24·3 |
| Great Field (old pasture) | 30. iii. 33 | 6·2 | 6·2 | 1·8 | 11·6 | 48·2 | 51·8 |

Because the determinations were made only on single sampling dates at different seasons no attempt is made to relate the results to the history of the fields. Their chief interest lies in showing that the values obtained on the Park Grass plots were similar to those of grassland soils under ordinary farm management, in spite of the somewhat artificial conditions of the Park Grass experiment.

The results of the Hoos Field experiment are given in full elsewhere (15). The soil was essentially an arable soil rather than a grassland one, and the level of mineralizable nitrogen was much lower than that in the Park Grass and other grassland soils. The mean value was 7·8 mg. N/kg. dry soil. Sampling extended only from 25 April to 6 July: over this period there was a falling off in mineralizable nitrogen similar to that observed during late spring and summer on the Park Grass plots. This may have been due in part to seasonal decomposition of soil organic matter combined with the removal of available nitrogen from the soil by the plants; but the values for $CO_2$ production suggested that another factor was the production by the maturing ryegrass of root material rich in cellulose, which would lock up some mineralizable nitrogen as it decomposed during incubation.

## D. Nitrification in Park Grass soils

It was pointed out in an earlier section that most of the Park Grass soils were consistent in producing predominantly either ammonia or nitrate on incubation. This is brought out in Table XI, in which nitrifiable nitrogen is expressed as a percentage of mineralizable nitrogen (or "percentage nitrate production"). The soils are arranged in order of increasing *p*H value.

The absence of major seasonal effects is shown by the high correlation between the 2 years. Some treatments were evidently less consistent than others as shown by the standard errors in the last column. It seems likely that the nitrifying organisms were unevenly distributed in the more variable plots, notably 13U. Limed plots always gave a greater pro-

portion of nitrate than the unlimed plots, and it is obvious from Table XI that the percentage nitrate production generally increased with increase in *p*H value. Treatment effects on nitrification appear to operate primarily through soil reaction but there are other special effects. Nitrate production was low relative to *p*H on the unmanured plots, limed and unlimed, and on the unlimed mineral plots (7 U) and high on plots 13 U (organic, unlimed) and 14 U (nitrate of soda + minerals, unlimed).

Table XI. *Nitrifiable nitrogen as a percentage of mineralizable nitrogen in Park Grass soils*

| Plot | Treatment | *p*H | 1932 Mean of 6 samplings | 1933 Mean of 7 samplings | 1932–3 Mean of 13 samplings |
|---|---|---|---|---|---|
| 9 U | S/A + minerals | 4·0 | 2 | −1 | 0 ± 0·8 |
| 13 U | Organic | 4·7 | 70 | 30 | 48 ± 9·8 |
| 9 L | S/A + minerals, limed | 5·0 | 49 | 24 | 36 ± 7·3 |
| 7 U | Minerals | 5·1 | 4 | 0 | 2 ± 0·9 |
| 3 U | Unmanured | 5·6 | 11 | 4 | 7 ± 2·7 |
| 14 U | N/S + minerals | 6·0 | 117 | 105 | 111 ± 2·5 |
| 13 L | Organic, limed | 6·6 | 104 | 98 | 101 ± 2·7 |
| 7 L | Minerals, limed | 6·7 | 126 | 110 | 117 ± 4·4 |
| 14 L | N/S + minerals, limed | 7·2 | 121 | 108 | 114 ± 2·8 |
| 3 L | Unmanured, limed | 7·3 | 82 | 93 | 88 ± 7·0 |

It appears that a deficiency of mineral nutrients (probably phosphate is the most important), or the absence of lime with no compensatory factor, depresses nitrification below the level to be expected from the soil reaction; whereas on the other hand the absence of lime can be compensated for by organic matter or nitrate of soda. There was no evidence that nitrogenous fertilizers in themselves had an adverse effect on nitrification, beyond their influence on soil reaction.

The extent to which the low nitrifying power of the most acid plots was due to a virtual absence of nitrifying bacteria, and to conditions in the soil unfavourable to their multiplication on incubation, required further study. D. W. Cutler and L. M. Crump (personal communication) examined the nitrifying activity of soil from plots 9 U and 9 L (sulphate of ammonia + minerals, unlimed and limed), by inoculating 10 g. of soil into 200 ml. of Winogradsky's medium; they obtained the following results for nitrite, mg. N/l.:

| | 9 unlimed | 9 limed |
|---|---|---|
| 17 days | None | Trace |
| 30 days | 1·6 | Very high |
| 11 months | 320 | — |

Enrichment cultures, taken when nitrite production was at a maximum and kept for several months, gave much nitrite with 9 L and little nitrite but much nitrate with 9 U.

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Thus the nitrifying organisms were not absent from these plots, but they were so few in 9U that a long period was required for them to become effective in a favourable medium.

### *Nitrification in other grassland soils*

The percentage nitrate production and the reaction of the soils discussed on pp. 99, 100 are given in Table XII, in order of increasing *p*H.

Table XII. *Nitrifiable N as percentage of mineralizable N, and reaction of grassland soils*

| Soil | Reaction *p*H | Nitrifiable N as % of mineralizable N |
|---|---|---|
| High Field | 5·1 | 55 |
| Great Field | 6·2 | 90 |
| Stackyard | 6·5 | 103 |
| Hoos Field | 8·0 | 117 |

The nitrification was obviously related to the reaction in spite of the great differences in the history of the soils. Indeed, the values for the first three soils, which were grazed and thus received dung, was closely comparable with the values for the dunged Park Grass plots (13U and 13L).

### *The disappearance of added nitrogen and the direct uptake of ammonia*

In connexion with the regular 4-weekly samplings of Park Grass soils it was remarked that nitrogen added as sulphate of ammonia or nitrate of soda soon disappeared from the soil (Figs. 1 and 3). Additional determinations not shown in those figures were made in order to follow the disappearance more closely. The full results, plotted on a logarithmic basis, appear in Figs. 7 and 8. The data for plot 9U are given separately in Fig. 7 because in January 1931, this contained much ammonia as a result of the damage received by the herbage in the frost of 1929. Some of this ammonia still remained when sulphate of ammonia was applied at the end of March, and the data for this plot are therefore shown as ammonia nitrogen actually present in the soil.

Fig. 8 also includes the results of the experiment on Stackyard Field, in which sulphate of ammonia was applied earlier in the season than on Park Grass. In this figure the data are expressed as *extra* nitrogen present in the soil, above a basal level which was either, (*a*) the amount in comparable unmanured plots (Stackyard), or (*b*) the average each year of the nitrogen-treated plots themselves, on sampling dates when none of the

values were influenced by adverse weather conditions or by recent applications of nitrogen (Park Grass).

The initial values in Fig. 8 were calculated from the rates of application. Witb a sampling depth of 20 cm., on Park Grass, the round figure of 40 mg./kg. is a close approximation to the amount of nitrogen applied in a dressing of 86 lb. N/acre as sulphate of ammonia, and 20 mg./kg. is the corresponding figure for the single half-dressing of nitrate of soda. The actual determinations recorded in Fig. 7 showed quite good agreement with this calculated value. On Stackyard Field, where the soil was more compact than on Park Grass, as it had recently been arable land, the round figure of 20 mg./kg. is taken for a rate of application of 0·4 cwt., or 45 lb., of nitrogen per acre.

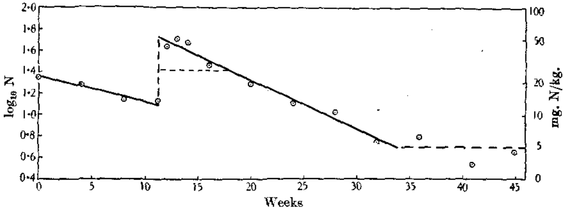


Fig. 7. Ammonia nitrogen in Park Grass soil, plot 9 U (sulphate of ammonia and minerals, unlimed), 1931. The ordinates are the logarithms of the mg. N/kg. dry soil. The vertical step represents the calculated addition of nitrogen in the sulphate of ammonia application.

It is clear from Figs. 7 and 8 that the disappearance of the nitrogen can be represented by straight lines on a logarithmic scale. It follows that under any given conditions the rate of disappearance was proportional to the amount of extra nitrogen that was present, while the time required for half of the nitrogen to disappear was a constant.

It is also evident in Fig. 8 that the average rate of disappearance of the nitrogen varied with the date of application, the disappearance being more rapid, the later the nitrogen was applied. This is an obvious result of the seasonal increase in soil and air temperature, with its effect on the activity of micro-organisms and plants.

To give some idea of the actual rates of disappearance of added nitrogen, the half periods obtained from the data in Fig. 8 are set out in Table XIII.

Althongh these figures are only approximations, they show none the

less how rapidly added nitrogen disappears from grassland soils, and how the rate of disappearance increases with the lateness of the application.

The mechanism of this disappearance remains to be considered. Nitrogen added to grassland soil may be taken up directly by plant roots, or it may be assimilated by micro-organisms and either liberated in another form (as in nitrification) or locked up in their tissues. Nitrate might also be washed away by rain, but there is abundant evidence from cropped lysimeter experiments that very little nitrate is actually lost in the drainage water of grassland soils.

Table XIII. *Disappearance of added nitrogen: half periods in days*

| Treatment | 6 Dec. 1929 | 17 Feb. 1930 | 26–27 Mar. 1931 | 28–29 Mar. 1933 | 26–27 Apr. 1932 |
|---|---|---|---|---|---|
| *S/A (Stackyard)* | 14 | *10* | — | — | — |
| S/A + minerals, limed (Park Grass) | — | — | 7 | 8 | 3 |
| N/S + minerals, unlimed (Park Grass) | — | — | 6 | 4 | 2 |
| *N/S + minerals, limed* (Park Grass) | — | — | 7 | 6 | 2 |

In none of the experiments shown in Figs. 7 and 8 was more than 1–2 mg. N/kg. of extra nitrate found in the soil after a sulphate of ammonia application; this is true also of other experiments not recorded here. An increase of up to 4 mg. N/kg. in ammonia was sometimes observed after nitrate of soda applications. This was unexpected, and it suggests that there may have been some excretion of ammonia from the grass roots back into the soil; but in any event the amount was not large. On the whole, little of the added nitrogen appeared in the soil in the alternative form: most of it was rapidly assimilated by micro-organisms or plants, whether or not nitrification took place as an intermediate stage with sulphate of ammonia.

On the question of nitrification, abundant evidence is available both in the present work and in that of Hall *et al.* (8) that nitrification is poor on the Park Grass plot 9L and absent on plot 9U (S/A + minerals with or without lime). Hall and his co-workers suggested as a result of their experiments that ammonia was taken up directly on these plots, and the results of the present work, in which the rate of disappearance of added ammonia was shown to be almost as rapid as that of added nitrate, makes this practically certain. As an additional test, the behaviour of added nitrogen in the soil in the presence and the absence of growing herbage was examined.

For this experiment the above-ground portion of the herbage was

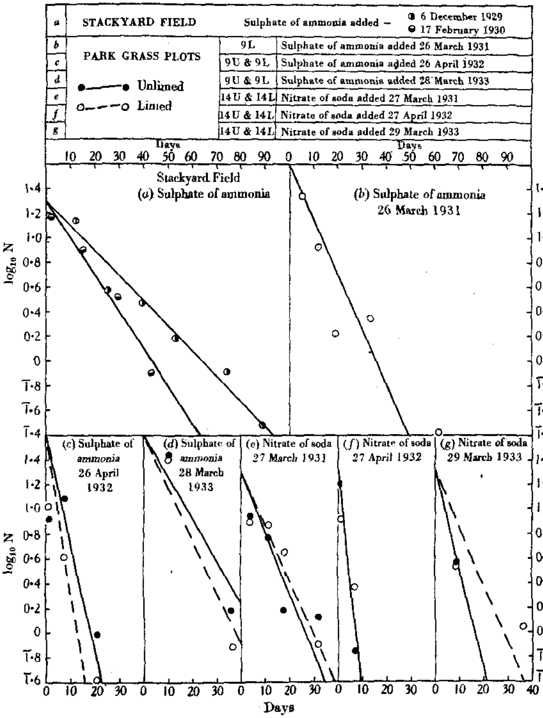


Fig. 8. Disappearance of added nitrogen from (*a*) Stackyard Field soil and (*b*) to (*g*) Park Grass plot soils. The ordinates give the logarithms of the excess of ammonia or nitrate nitrogen (as mg. N/kg. dry soil) above the normal values. The abscissae give the intervals since the dates of application of nitrogenous fertilizer, which are shown in each diagram.

completely removed from three separate square-foot areas on each of plots 7 U and 7 L (minerals only), 9 U and 9 L (S/A + minerals) and 14 U and 14 L (N/S + minerals). Each of the bare areas was isolated from surrounding herbage by a vertical spade-cut to a depth of 20 cm. on each side, and a horizontal spade-cut was made about 2–4 cm. below the surface to sever all roots. The turf was lightly trodden down, the bare area was covered with cellophane to prevent leaching, and any green shoots that appeared subsequently were removed.

The herbage was removed on 29–30 March 1933, one day after the application of sulphate of ammonia and nitrate of soda respectively. At the same time a sample was taken from the soil surrounding each square; each sample, consisting of four cores to a depth of 20 cm., was analysed separately. Twenty-two days later the soil of the bare areas was sampled similarly, and also that under the growing herbage around each square. The results are given in Table XIV.

Table XIV. *Influence of growing herbage on the behaviour of added nitrogen*

| | | Ammonia (mg. N/kg.) | | | | Nitrate (mg. N/kg.) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 29–30 Mar. | 20–21 Apr. | | | 29–30 Mar. | 20–21 Apr. | | |
| Plot | Treatment | Under herbage | Bare soil | Under herbage | S.E. (±) | Under herbage | Bare soil | Under herbage | S.E. (±) |
| 7 U | Minerals | 4·8 | 6·9 | 3·3 | 0·89 | 0·7 | 1·5 | 0·8 | 0·13 |
| 7 L | Minerals, limed | 5·3 | 6·4 | 4·8 | | 0·5 | 1·5 | 0·9 | |
| 9 U | S/A + minerals | 24·4 | 37·2 | 6·2 | 6·7 | 0·7 | 1·4 | 0·9 | 0·60 |
| 9 L | S/A + minerals, limed | 21·5 | 27·0 | 5·7 | | 0·9 | 2·9 | 1·1 | |
| 14 U | N/S + minerals | 4·1 | 5·4 | 4·1 | 0·44 | 16·9 | 10·8 | 1·8 | 4·6 |
| 14 L | N/S + minerals, limed | 4·2 | 4·4 | 4·2 | | 17·3 | 13·6 | 3·4 | |

Sampling errors were high in the presence of added nitrogen, because of the limited numbers of positions sampled.

The results on the control plots, 7 U and 7 L, showed that the damage to the herbage involved in the treatment of the bare areas did not in itself cause much change in the soil nitrogen. There was a slight rise in ammonia content, giving a significant average increase compared with the soil under herbage at the end of the experiment, and a small but significant rise in nitrate content.

When sulphate of ammonia was added (plots 9 U and 9 L) the extra nitrogen almost completely disappeared where the herbage was growing, but it remained in the soil as ammonia in the bare areas. The apparent increase was not significant. There was a slight increase in the nitrate content of the bare soil in both plots, but only that on the limed plot was

significant. The rate of nitrification in the bare soil was entirely negligible in comparison with the rate of disappearance of the added ammonia under herbage.

The conclusion is inescapable that the disappearance of the added ammonia under growing herbage was due to its direct uptake by the herbage.

With nitrate of soda (plots 14U and 14L) there was (*a*) a slight increase in ammonia content in the bare soil, significant only on plot 14U; (*b*) almost complete removal of the added nitrate under herbage; and (*c*) an apparent decrease in nitrate content, not sufficient for significance, in the bare areas.

While it is thus clear that added ammonia, no less than added nitrate, was taken up directly on the Park Grass plots, the question arises whether this is true on other soils. In experiments on Great Field (old pasture) and on Hoos Field (temporary ley) the writer has found such rapid disappearance of nitrogen added as sulphate of ammonia in April or May (half-period approximately 4 days in each experiment) as to make it unlikely that much nitrification took place before the herbage obtained its nitrogen. In the Hoos Field experiment a response to the nitrogen was, indeed, visible in the herbage a week after application. In the Stackyard Field experiment (Fig. 8) the disappearance of added ammonia nitrogen was slower, but the herbage was almost dormant at the times when sulphate of ammonia was applied. Eggleton (17), who analysed both soil and herbage after the application of various forms of nitrogen, found a large increase in herbage ammonia but little increase in nitrate when sulphate of ammonia was used on the Jealott's Hill soil.

It is probably generally true that when sulphate of ammonia is applied to a grassland soil, much if not most of the ammonia is taken up directly without previous nitrification. With the soil so completely occupied by roots, this direct absorption is facilitated and there is little time for nitrification of the ammonia to occur. In acid soils where nitrification is poor, the entire uptake of nitrogen, whether from added sulphate of ammonia or from the decomposition of the soil organic matter, will be in the form of ammonia rather than nitrate. It is not precluded, however, that some of the nitrogen from animal excreta or decomposing organic matter may be taken up in more complex soluble forms such as urea, asparagine, or amino-acids.

A question not so far examined is that of the proportion of the added nitrogen which is secured by the herbage, and the proportion which remains in the soil, probably held by micro-organisms. In experiments

on Stackyard and Sawyers Fields (Richardson (3)) the "recoveries" of nitrogen in the herbage after early spring applications of sulphate of ammonia (increase over control plots in the first one or two cuts) were 36 and 38 % respectively. These were young grassland soils (2 or 3 years old) and the low recoveries may have been associated with the locking up of nitrogen which takes place in such soils as their nitrogen content increases.

In the Park Grass experiment, calculation of the recovery of nitrogen on the nitrogen-treated plots is complicated by differences in herbage between these and the no-nitrogen "control", the latter containing Leguminosae which are almost or entirely absent from the former. It may be questioned, however, whether a comparison with "control" plots is necessary. Since Leguminosae are absent from the nitrogen-treated plots, the whole of the nitrogen found in the grasses must have come from the soil, and it will represent the nitrogen added in the fertilizers along with any fixed by free-living micro-organisms. On the acid sulphate of ammonia plots at least the latter quantity is likely to be small, for *Azotobacter* is absent from these plots (Ziemięcka (31)).

Table XV. *Nitrogen in herbage of nitrogen-treated plots, Park Grass*

lb./acre

| | | 1931 | | | 1932 | | | 1933 Only | |
|---|---|---|---|---|---|---|---|---|---|
| Plot | Treatment | 1st cut | 2nd cut | Total | 1st cut | 2nd cut | Total | 1st cut | Annual mean |
| 9U | Sulphate of ammonia and minerals | 91·3 | 26·4 | 117·7 | 86·3 | 9·8 | 96·1 | 64·4 | 92·7 |
| 9L | | 81·3 | 28·7 | 110·0 | 80·6 | 21·9 | 102·5 | 82·0 | 98·2 |
| 14U | Nitrate of soda and minerals | 72·1 | 28·2 | 100·3 | 63·6 | 26·5 | 90·1 | 79·0 | 89·8 |
| 14L | | 66·5 | 23·9 | 90·4 | 87·7 | 21·0 | 108·7 | 72·8 | 90·6 |

This table shows the unexpected result that more nitrogen was recovered from the highly acid sulphate of ammonia plots than from the nearly neutral nitrate of soda ones, while in all plots the average amount removed from the soil over the 3 years was more than the 86 lb. per acre applied annually in the treatments. On plot 9U some extra ammonia was present in the soil at the beginning of 1931, which would explain the very high value for that year; on plots 14U and 14L the difference between the annual mean and the nitrogen added is of the same order as the amount of nitrogen present in a year's rainfall, 4 lb. per acre (Hall (18)). On plot 9L, however, 8 lb. more nitrogen than this was recovered in the herbage. Since the mean of only 3 years is being considered and one of these gave only one cut of hay, it would be unwise to attach too much

weight to differences of this order; the results show at least that a large proportion of the added nitrogen was recovered in the herbage on these old grassland soils.

Another indication that most of the added nitrogen was taken up by the herbage appears in the experiment (p. 106) on the effect of removing the herbage: there was no significant disappearance of added nitrogen from the soil unless plants were actually growing on it.

### E. THE TOTAL NITROGEN CONTENT OF GRASSLAND SOILS

It is to be expected that the total nitrogen content of grassland soils must approach some equilibrium value controlled by environmental conditions. Jenny (19) has already shown that for a wide range of roughly comparable grassland soils in the United States the nitrogen contents can

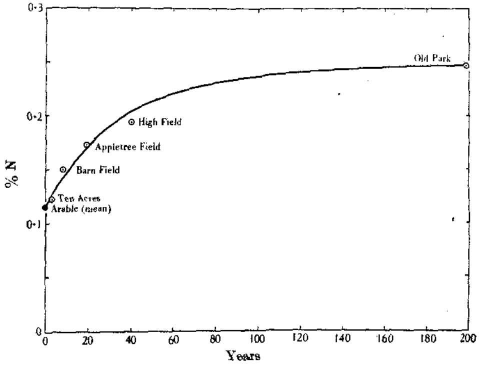


Fig. 9. Relationship between total soil nitrogen and age of grassland at Rothamsted.

be expressed as a function of the climate, increasing exponentially with rise in humidity and fall in temperature. The rate of approach to this equilibrium would be difficult to determine for a single field, but some information can be obtained by re-examining data for grassland fields at Rothamsted given by Lawes & Gilbert. The analyses were by the soda lime method which gives lower results than the Kjeldahl method. Excluding "Dr Gilbert's meadow", which received heavy annual dressings of dung and was managed under unusual conditions, the data

in Fig. 9 show that the rate of increase in nitrogen falls off steadily and to a first approximation may be taken as proportional to the deficit below a final maximum which is assumed to equal that of the Old Park (0·2466 % nitrogen) by the soda lime method. In the equation $dx/dt = k\ (a - x)$ the value of $K$ (0·028) is such that the nitrogen content would move half-way to the equilibrium in about 25 years.

On the Park Grass plots it may be assumed that when the experiment began the nitrogen content of the soil was at equilibrium and fairly uniform over the field (since the land had been under grass for centuries and was uniform to the eye). The influence of treatment on the nitrogen content was studied by the analysis of bulked material from the samples taken for incubation over the period August–December 1932. The carbon content was also measured by the Bangor wet combustion method (20), the determinations being kindly made by L. W. Raymond. The results in Table XVI are based on duplicate subsamples, which generally agreed closely.

Table XVI. *Carbon and nitrogen content of Park Grass soils*

| Plot | Treatment | Carbon % | Nitrogen % | C/N ratio |
|---|---|---|---|---|
| 3U | Unmanured | 3·60 | 0·288 | 12·5 |
| 3L | Unmanured, limed | 3·76 | 0·314 | 12·0 |
| 13U | Organic | 2·97 | 0·250 | 11·9 |
| 13L | Organic, limed | 3·45 | 0·280 | 12·4 |
| 7U | Minerals | 2·70 | 0·235 | 11·5 |
| 7L | Minerals, limed | 3·06 | 0·266 | 11·5 |
| 9U | S/A + minerals | 3·91 | 0·302 | 12·9 |
| 9L | S/A + minerals, limed | 3·87 | 0·309 | 12·6 |
| 14U | N/S + minerals | 3·10 | 0·265 | 11·6 |
| 14L | N/S + minerals, limed | 3·58 | 0·297 | 12·1 |
| S.E. | | ±0·054 | ±0·0024 | |
| Mean of unlimed plots | | 3·26 | 0·268 | 12·1 |
| Mean of limed plots | | 3·54 | 0·293 | 12·1 |

The carbon and nitrogen content of the soils was in general closely related, with only small fluctuations around a mean C/N ratio of 12·1. Thus the nitrogen content was a fair index to the total organic matter content of the soils.

Although liming had on the average no effect on the C/N ratio, it had a striking action on the actual carbon or nitrogen content. With every pair of similarly manured plots, the limed plot had a higher nitrogen content than the unlimed one; with all pairs but one (9U and 9L) the carbon content was also higher in the limed plot.

It has sometimes been stated that liming causes a loss of organic matter from the soil by accelerating microbiological action. In these

grassland soils there was, on the contrary, not a loss but a gain in organic matter with liming. It appears that under the equilibrium conditions of grassland soils any acceleration of the down-grade side of the cycle caused by liming was more than counterbalanced by an acceleration of the up-grade processes.

Probably related to the greater organic matter content of the limed plots was the fact that the water content under field conditions was consistently higher in the limed plots (see Fig. 2, p. 82).

The influence of individual treatments on nitrogen content is the result of such a complex of factors—yield and type of herbage, worm action, microbiological action—that interpretation in detail can be little more than guesswork. Some guide is available in the results for mineralizable nitrogen. The plots receiving organic manures (13 U and 13 L) were, unexpectedly, lower in total N than the unmanured plots (3 U and 3 L): in the incubation experiments mineralizable nitrogen was found to be much higher with organic manures (p. 96), while worms were also more active (p. 114), and it appears that the down-grade side of the cycle was accelerated to an extent more than sufficient to decompose the nitrogen added in the organic matter.

The plots receiving minerals only (7 U and 7 L) were lower still in total soil nitrogen—they were the lowest, in fact, in their respective groups (limed or unlimed)—and in their soils mineralizable nitrogen was also higher than in the unmanured plots. Plots 7 U and 7 L must gain some nitrogen from the high proportion of leguminous herbage present, but this apparently was not sufficient to counteract the acceleration of microbiological breakdown caused by the mineral nutrients.

Plots receiving nitrogenous fertilizers in addition to minerals contained more soil nitrogen than the corresponding "minerals only" plots, the increase being greater with sulphate of ammonia than with nitrate of soda. This may be related to the fact that the mineralizable nitrogen was less with sulphate of ammonia: in other words, the down-grade side of the cycle was less active, and the equilibrium total nitrogen level correspondingly higher.

The unlimed sulphate of ammonia plot, which had a "mat" of undecomposed organic material at the surface, was little higher in carbon and no higher in nitrogen than the limed plot. Along with the "mat" there was a deficiency in organic matter below the surface layer; in the limed plot the organic matter was more evenly distributed but the same total quantity was present in the soil. A similar result has been recorded by Powers (21) in comparing podzolized and brown forest soils: although

the podzolized soil appeared richer in organic matter at the surface, the total amount in the profile was the same in both soils.

In spite of the regular effect of liming in raising the nitrogen content of the Park Grass soils, reaction as such did not appear to be very important: the correlation with all treatments ($r = +0{\cdot}60$) was not significant.

*Worm action in grassland soils*

Darwin, in his well-known book, *Vegetable Mould and Earthworms*, showed the great importance of worm action in soil formation, but the implications of their activity tend to be overlooked. In grassland soils earthworms are responsible for most of the cultivation and much of the aeration and drainage of the soil; for dragging down vegetable fragments which would otherwise remain on the surface; and for "manuring" the surface with their casts, which are relatively rich in organic matter and available nitrogen. By this action they eventually build up a new soil on the surface of the old; most of the soils sampled in the present investigation were, in fact, a mass of weathered and consolidated worm casts.

This was true of Park Grass where the examination of a profile pit showed at the top about 20 cm. of crumbly soil almost free from stones, and then a definite layer of scattered flint pebbles evidently representing the surface of old arable land from which the upper layer of earth had been transported by worm action extending over several centuries. In Great Field, which had been 59 years under grass, there was an upper layer of crumbly, stoneless earth about 10 cm. thick, and below this a very strongly marked bed of pebbles. The thickness of the layer of fine earth on Great Field corresponds with an average rate of deposition of 1 in. in 15 years, which is rather less than Darwin's estimate of 1 in. in about 10 years. On Park Grass the average rate would be still less. It must be remembered, however, that much of the activity of the worms is restricted to the top few inches of soil, so that as time went on and the upper layer of soil derived from casts became deeper less material would be brought up from below: the rate of increase in thickness would consequently fall off with time.

With these observations in mind it is possible to form a clearer picture of what takes place when arable land is put down to grass and left for a long period of years. In addition to the multiplication of roots in the soil, which will tend to cause some increase in organic matter and nitrogen content, there is the production by worm action of a surface layer of gradually increasing depth, considerably richer in nitrogen than the soil

below. Bates (22) and others have recorded analyses of soils and the associated worm-casts: the actual nitrogen contents varied with the nature of the soil, but the worm-casts always contained more nitrogen.

Thus the approach to an "equilibrium" nitrogen content in a grassland soil depends not simply on a gradual increase in organic matter throughout the soil, but also on the progressive growth in thickness of the nitrogen-rich layer of worm-casts. The falling-off with time observed in the rate of increase of the nitrogen content may result both from an increase in the intensity of the down-grade side of the cycle as organic matter accumulates, and from a falling-off in the rate at which the thickness of the top layer increases. The final equilibrium is reached when there is no further appreciable increase in the thickness of the layer of fine earth, and the rate of loss of organic matter by decomposition is equal to the rate of gain in organic matter both from root residues and from the plant residues incorporated in their casts by the worms.

In view of the important part played by earthworms an attempt was made to estimate their activity in the Park Grass soils. As it was not possible to take a sufficient number of large soil samples for a count of actual worms, the worm-casts were counted instead. While this does not give a direct measure of worm numbers it does measure their activity, subject to the possibility of the average size of casts varying with treatment. Actually there appeared to be little difference in the average size of the casts except on plot 13 L, where large ones were noticeably more abundant.

The counts were made on 26 and 28 November 1934, by one observer on the former date and by two others on the latter. Each observer counted the casts in nine random throws of a 1 ft. quadrat on each plot. In addition to the ten plots sampled in the soil investigations, another unmanured, unlimed plot was included (plot 12, adjacent to treatment 13 and at the other end of the field from treatment 3), and counts were also made in the adjacent parkland of High Field. The results follow in Table XVII, the standard error per plot being based on the variation between individual quadrat throws.

The most striking result is the complete absence of worm-casts from the very acid, unlimed, S/A + minerals plot (9 U). This, taken with the considerable amount of mineralizable nitrogen produced on incubation, makes it clear that the formation of the mat on this plot was due to an absence of worms and not simply to a degree of acidity unfavourable to the decomposition of organic matter.

On the other plots treated with artificial fertilizers (with or without

nitrogen) the number of casts was of the same order as the number in the adjoining grazed parkland (High Field), and usually less than on the unmanured plots. Plot 7 L (minerals, limed) gave more casts than the other plots receiving fertilizers, the number being about the same as on the unmanured plots. It is worthy of comment that in spite of almost 80 years' continuous treatment with heavy dressings of mineral salts the activity of the worms had not been depressed.

Table XVII. *Worm-casts on grassland soils*

| Plot | Treatment | Number of casts (thousands per acre) |
|---|---|---|
| 12 | Unmanured | 294 |
| 3 U | Unmanured | 245 |
| 3 L | Unmanured, limed | 276 |
| 13 U | Organic | 423 |
| 13 L | Organic, limed | 337 |
| 7 U | Minerals | 136 |
| 7 L | Minerals, limed | 252 |
| 9 U | S/A + minerals | 0 |
| 9 L | S/A + minerals, limed | 127 |
| 14 U | N/S + minerals | 153 |
| 14 L | N/S + minerals, limed | 161 |
| High Field | Grazed | 178 |
| S.E. | | ± 26·4 |

The three unmanured plots contained similar numbers of worm-casts, although plot 12 was separated by almost the length of the field from the others. Organic manures, on plots 13 U and 13 L, gave the highest counts, as might have been expected.

The limed plots generally gave more casts than the unlimed plots, which was also in accord with expectation, although the difference was negligible on the nitrate of soda plots, and with organic manures more casts were actually found on the unlimed plot. Moles were very active on the limed organic plot, and they had doubtless reduced the numbers of worms.

It was rather striking that there should be so much worm action on the unlimed plots, especially 7 U, for some of these soils were highly acid. Only the extreme degree of acidity found on plot 9 U was sufficient to inhibit worm action completely. There was no general relation between numbers of casts and soil reaction, the correlation coefficient ($r = +0{\cdot}35$) being far below significance.

To obtain an estimate of the relationship which the worm-casts on the Park Grass experiment bore to the soil as a whole, fifty casts were collected from plot 13 L and weighed. The dry weight per cast of 2·72 g. dry soil corresponds with about 1 ton of worm-casts per acre. Since the number was higher on plot 13 L than on most of the others, and the casts were

possibly somewhat larger, the weight of casts on other treatments would be less than this. Even if it were taken at about ½ ton per acre, this is an appreciable "manuring" of the soil, for it is doubtless repeated many times in the course of the year. Darwin gives estimates of from 7 to 18 tons per acre as the total amount brought up in worm-casts in a year.

The relation between worm activity and the nitrogen cycle in the Park Grass soils is a complex one. Apart from the general observation that both worm-casts and such quantities as total nitrogen, ammonia nitrogen, and nitrification tended to be higher on the limed than on the unlimed plots, no relationship existed between the counts of worm-casts and the other measurements on the individual plots. With nitrification, for example, which might be expected to be favoured by similar factors to worm activity, the correlation was not significant ($r = +0{\cdot}41$). In an arable soil newly laid down to grass greater worm activity would cause a more rapid increase in the thickness of the layer of casts, and so a more rapid increase in nitrogen content: but in a mature grassland soil the aeration and mixing effected by the earthworms might accelerate the decomposition of organic matter to an extent sufficient to offset the gain from the casts deposited on the surface.

No actual counts of worms in grassland soils at Rothamsted are available for comparison with the numbers of casts. In arable soils Morris (23) found numbers varying between nil and 960,000 per acre on Barn Field, and between 458,000 and 1,010,000 per acre on Broadbalk. The higher numbers were in farmyard manure plots on both fields. The numbers of casts found on Park Grass were thus of the same order as the numbers of worms in the arable soils. Thompson (24) at Aberystwyth counted the worms in pasture grassland at fortnightly intervals over 2 years, and the numbers given, reduced to an acre basis, averaged about 250,000 worms per acre. K. D. Baweja (25) counted 3,000,000 per acre in Great Field at Rothamsted, as the average of thirteen monthly samples. Many of the worms were very small ones.

## DISCUSSION

It is clear from the foregoing work that the condition of the nitrogen in grassland soils in a temperate climate can usefully be pictured as a series of equilibria. These are not the simple reversible equilibria of physical chemistry, but dynamic equilibria between the production and the removal of the forms of nitrogen found at successive stages of the cycle. In a system exposed to such a range of uncontrolled variables as a

grassland soil, the "equilibria" are not as definite or constant as those *studied in the laboratory, but the fluctuations and irregularities which occur are of a relatively minor order.*

The equilibrium levels of the various forms of nitrogen differ greatly, as do the rates at which equilibrium is reached. The level of total nitrogen is many hundred times that of mineral nitrogen or ammonia nitrogen, while the half period for reaching equilibrium in total nitrogen is of the order of 25 years. On the other hand, the half period for ammonia or nitrate nitrogen is only of the order of a few days, or at most, in winter time, 1 or 2 weeks.

In young grassland the equilibrium level of mineral nitrogen is subject to change, for as the amount of organic matter in the soil increases and the total nitrogen content rises towards the level for old grassland, the mineral nitrogen also increases. This is, however, a long-period change which does not prevent the existence of temporary equilibrium values over shorter periods. Over still longer periods, of the order of geological time, the total nitrogen is doubtless liable to change also: changes in climate, or alterations in composition of the soil resulting from pedogenic processes, would of course lead to changes in the nitrogen equilibrium. It is unlikely, however, that a soil would remain under grass for such long periods except where steppe conditions obtained. Steppe or prairie soils are, of course, the ultimate expression of grassland, but they are not formed in the temperate, oceanic, climate of England.

Since the equilibrium levels found in the present work were those existing under Rothamsted soil and climatic conditions, other levels would be expected under different conditions elsewhere. Reference has already been made to Jenny's work on the influence of temperature and moisture on the total nitrogen of loamy grassland soils. Soil texture must also be important, for it is general experience that organic matter decays away more quickly in a sandy soil than in a heavy one: the down-grade side of the cycle is more active, and lower total nitrogen equilibria, with possibly higher mineral nitrogen equilibria are to be expected. An examination of the results of Kay (26) for a series of soils of varying texture existing under a fairly uniform type of climate showed such a relationship to exist, the total nitrogen content of old grassland soils being greater the higher the proportion of clay in the soil.

The level of mineral nitrogen (that is, ammonia as well as nitrate) under grassland soils has received little attention elsewhere, except at Jealott's Hill in work contemporaneous with but independent of the present investigation. There, with a rather lighter soil and lower rainfall

than at Rothamsted, Eggleton (17) obtained generally similar results. His values are not directly comparable, for they refer to a sampling depth of 10 cm., which would in any case give higher nitrogen levels than the 20 cm. used in the present work, and would probably also accentuate fluctuations. He found that the nitrate level (usually 2–3 mg. N/kg.) was almost always below the ammonia level (usually 5–15 mg. N/kg.), but the values of both tended to be higher and to fluctuate more than at Rothamsted. Eggleton likewise observed the rapid disappearance of added ammonia, and found that it was more rapid in early June than in mid-March. In later papers he showed that part at least of the ammonia could be found in the herbage shortly after application.

Some isolated determinations of ammonia and nitrate in grassland soils in Victoria (Australia) were reported by Penman (27), who related them to the response of the grassland to nitrogenous fertilizers. Full experimental details were not given, but the determinations do not appear to have been made on fresh samples, and the very high values reported may have resulted from storage or drying. Nitrate was from 1 to 3 mg. N/kg.; ammonia from 11 to 42 mg. N/kg., in samples of 15 cm. depth. Still higher ammonia, 87 mg. N/kg., was found in "rank patches" where stock had urinated.

Nitrate, but not ammonia, levels under timothy sod or other forms of grassland were studied by Lyon, Bizzell and other American workers (28). They agreed in finding always low concentrations (of the order of a few pounds per acre) of nitrate in grassland soils or drainage waters, and rapid disappearance of nitrate added as nitrate of soda.

A pasture soil near Danzig was studied by Stremme & Schroedter (29), who took monthly samples from April to September; they found nitrate nitrogen was always low (1·5 mg. N/kg. or less), but ammonia nitrogen was relatively high in April and May (12–15 mg./kg.) and lower (3·6–4·8 mg./kg.) from June to September. The high values in spring may have been associated with the damage to the herbage caused by a winter of the "continental" type.

Because the subject of the present investigation was the nitrogen cycle in the soil, the question of the part played by grazing animals, which is of course an important aspect of the use of grassland, has so far been excluded. Their direct participation in the nitrogen cycle may be regarded as a matter of accelerating the conversion of herbage to soil organic matter and available forms of nitrogen. Milking or growing stock will remove some nitrogen from the land, while stock supplied with additional food out of doors will add some extra nitrogen. Management

of the grazing factor may cause changes in the herbage which, by leading to an increase or decrease in Leguminosae, will affect the amount of nitrogen fixed from the air.

However, it has been apparent in the present work that the differences between otherwise similar grassland soils caused by grazing or the addition of animal excreta are not large. Great Field soil, an old pasture, behaved generally similarly to Park Grass soil, which has been continuous meadow for many years. The soils from plots on Park Grass receiving quite heavy dressings of organic manures (including 14 tons per acre of farmyard manure every 4 years) were not markedly different in general behaviour from other soil receiving heavy dressings of artificials. The difference between grassland and arable soils is a much more fundamental one than the difference between meadow and pasture.

The function of grassland in general agriculture, that of raising the fertility of the soil, is too large a question to discuss fully here, but the bearing of results obtained in the present investigation may be indicated. In so far as increased fertility is due to an increased supply of nutrients—of which nitrogen is, in this country, the most important—the investigation has shown that both the immediately available nitrogen (mineral nitrogen in fresh soil) and the potentially available nitrogen (of which mineralizable nitrogen produced on incubation is an index) are higher in old grassland soils than in a temporary ley. An expression for the rate of accumulation of total nitrogen has been worked out, which showed how slowly this accumulation takes place: it is probably in great part a matter of building up of a new layer of soil by worm-casts. All these considerations point to the need for land being put down to grass for long periods if any prolonged gain in fertility is to result: it is doubtful whether a 1-year ley would do much more than counteract the normal wastage of organic matter and nitrogen during a rotation.

So far "fertility" has been considered in terms of nitrogen, but other factors such as water-holding capacity and soil structure also run parallel with the increase in organic matter that takes place under grassland. The difference in structure between grassland and arable soils is well shown by Savvinov (30), who found on samples taken by the writer that in Park Grass plots 76–83 % of the soil was in structural units larger than 1 mm. diameter, whereas on Broadbalk there was only 22 % in the farmyard manure plot and still less (6 %) in the unmanured plot.

SUMMARY

1. In an examination of Park Grass soils extending over 3 years, and in shorter studies of other grassland soils, the fresh soil always contained more ammonia than nitrate nitrogen. The levels of both were low, and in spite of minor fluctuations were sufficiently constant to suggest the existence of equilibrium conditions in the nitrogen cycle. Climatic factors had no appreciable influence except on one very acid plot; here frost or drought, severe enough to kill the herbage, allowed ammonia to accumulate. Otherwise, treatment had little effect, but liming tended to give higher ammonia and lower nitrate levels. The equilibrium levels of both ammonia and nitrate were higher in old grassland soils than in land newly put down to grass.

2. "Mineralizable" nitrogen, produced by incubating the fresh soils under standard conditions, showed a seasonal rhythm the opposite of the annual temperature rhythm, tending to a maximum in winter and early spring and a minimum in summer and early autumn. This was related to the addition and decay of organic residues in the soil. An abnormally dry summer caused a temporary rise, especially on the more acid plots. An extremely acid plot, on which a mat accumulated in the field, produced as much mineralizable nitrogen on incubation as more normal soils.

3. Ammonia and nitrate production on incubation differed greatly with different treatments, apparently as a result of the influence of treatment on soil reaction. The more acid soils, with $p$H less than 6·0, produced chiefly ammonia, while the less acid soils produced chiefly nitrate. There was a significant correlation between reaction and percentage nitrification.

4. Nitrogen added as sulphate of ammonia or nitrate of soda rapidly disappeared, the half period being of the order of a week or two in winter or early spring and a few days in late spring. The rapid disappearance of ammonia, even on plots in which nitrification was poor or lacking, suggested that it was being taken up directly by the herbage, and this was confirmed in an experiment in which added ammonia was found to remain in the soil when the herbage was removed but to disappear rapidly under normal herbage.

5. The total nitrogen content under Rothamsted conditions increased with age, when arable soil was laid down to grass, the time required for the nitrogen to move half-way to the equilibrium content (that of very old grassland) being about 25 years. In the Park Grass experiment liming was found to increase the nitrogen, and the carbon contents of the soil.

Plots receiving organic manures (including farmyard manure every 4th year) were lower in nitrogen and carbon content than the unmanured plots. A count of worm-casts showed the highest numbers on the organic manured plots, while limed plots usually contained more than unlimed. Worms were absent from the extremely acid, matted, plot already mentioned. Thus the formation of the mat was due to the effect of acidity on the worms, rather than on the microbiological decomposition of the organic matter.

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# FECUNDITY OF MALE RABBITS AS DETERMINED BY "DUMMY MATINGS"

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(With Plate II and Four Text-figures)

## INTRODUCTION

A QUANTITATIVE method of estimating the fertility of the male is of great value in both human clinical practice and in animal husbandry. In a previous paper (Walton, 1927) the theory relating fertility with the number of spermatozoa produced by the male was discussed, and the conclusion drawn that potential fertility or the probability of fertilization was a direct function of the number and viability of the spermatozoa. That sperm counts may prove valuable in the diagnosis of male fertility has been shown by Walton & Fair (1928), Williams & Savage (1925), McKenzie & Berliner (1937) and others. But much work under controlled experimental conditions is still required to establish norms, and elucidate some of the causes of divergence from the norm due possibly to age, nutrition, endocrine disturbance and genetic variability.

It seemed important therefore to investigate the number of spermatozoa normally produced by male rabbits under experimental conditions and to study the variability in different circumstances.

## TECHNIQUE

The use of a "dummy" fitted with an artificial vagina is now an established method for the collection of semen for artificial insemination for both experimental purposes and practical application to animal husbandry (Milovanov, 1936; *Imp. Bureau Animal Genetics*, 1933).

The apparatus (artificial vagina) used by Kardymovich & Milovanov (1933) for the rabbit, was not available when we started our experiments. From their description, however, we had a model made as near to the original as possible. This proved cumbersome, and after some little experimentation was abandoned for a somewhat similar apparatus of our own. The apparatus and the method of heating it before use is represented diagrammatically in Text-fig. 1.

The artificial vagina consists of a glass[1] cylinder 8 cm. long and 3 cm. diameter. At one end is a rubber stopper through which pass two glass tubes with rubber connexions (*D* and *E*). At the other end a piece of thick rubber tubing (*A*) covers the edge of the cylinder. A thin rubber sheath (*B*) 12 cm. long, at one end 3 cm. in diameter, and tapering at the other end to $1\frac{1}{4}$ cm., passes down the centre of the cylinder. The

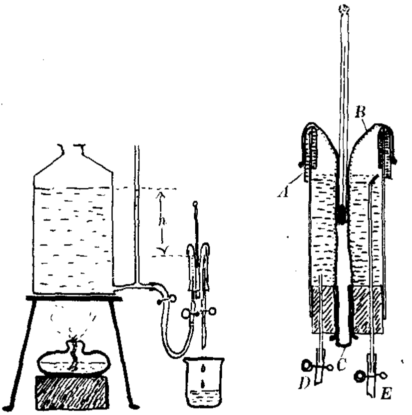


Fig. 1. For description see text.

wide end of the sheath is turned back over the rubber tubing *A* and held in position by a rubber band 2 cm. from the top on the outside. The narrow end of the sheath is passed through a hole of the same diameter in the rubber stopper. A small glass specimen tube (*C*) fits inside the sheath, and is just wide enough to hold the sheath in position and to be securely held by the pressure of the stopper. Round the outside of the whole apparatus fits a thick felt covering (not shown in the drawing).

When in use the apparatus is first connected to a supply of hot water as shown in the diagram. On opening the clips, hot water first fills the cylinder through the tube *D* until it reaches the level of the top of tube *E*. A certain amount of air is thus left in the apparatus. When the tem-

[1] Since this paper went to press a vulcanite cylinder has been used and found more satisfactory.

perature inside the sheath registers about 45° C., tube *E* is closed. The pressure in the water jacket now rises, closing the lumen of the sheath. By adjusting the height of the cylinder to a definite water level (*h*), the pressure which experience shows to be best can be maintained constant from one trial to another. When the pressure is adjusted *D* is closed and the cylinder is detached and ready for use.

The "dummy rabbit" consists of a rectangular piece of thick felt covered with rabbit skin about 40 cm. long by 30 cm. wide. The two long edges are tied together with tape to form a muff, into which the right hand of the operator holding the artificial vagina is inserted. The end of the muff which represents the hind end of the dummy rabbit is sewn together, leaving only a small aperture through which the artificial vagina just protrudes. It is important that the fur should fit firmly round the rim of the artificial vagina so that it is not displaced by the male when making copulatory movements. The correct height and angle at which the artificial vagina must be held in order to induce copulation and ejaculation is a matter of experience, and depends upon the skilful manipulation of the apparatus by means of the operator's hand within the muff. Before presenting the dummy to the rabbit, the inside of the sheath is smeared with vaseline by means of the thermometer and the temperature again read. This should register nearly 45° C. On presenting the dummy to the male, care should be taken that the sheath at the rim of the apparatus is kept warm by occasionally inverting the artificial vagina so that the warm water comes in contact with the rim. If the buck is responsive, copulation quickly follows. The penis is introduced into the opening provided by the sheath; ejaculation occurs and the semen is collected in the glass receiver. The apparatus in use is shown in Plate II.

The sample of semen is now taken to the laboratory for examination and estimation. First a small drop of semen is examined under the microscope to observe the motility and approximate density of the sperm. The semen of the rabbit consists of the sperm from the vas deferens, together with a milky fluid containing small granules from the prostate and a very variable quantity of clear secretion from the uterus masculinus which, on ejaculation, coagulates to form a jelly. This jelly-like coagulum has, in our experience, only been present in a few cases. Since its inclusion in the sample renders immediate dilution difficult, the procedure has been to remove it and not include it in the total volume of the ejaculate. The volume of the remaining fluid is now measured and the sample diluted for counting. After experimenting with several kinds of diluents the one finally adopted as the most suitable was NaCl 0·9 %.

Dilutions of 1 in 10, 1 in 100 and 1 in 1000 are usually made. Further dilution is seldom required. It is important to use the whole volume of the semen in making the first dilution, since large errors are involved if a smaller quantity is used. In subsequent dilutions at least 1 c.c. should be used if possible. A dilution which gives approximately five spermatozoa per square in the counting chamber, gives the most accurate results.

Ten minutes' interval is allowed for the fluid to settle down in the counting chamber before the count is taken. The technique of estimation and the errors of sampling have been described by Walton (1927). The accuracy of the count increases with the number of samples examined and with the number of spermatozoa counted, but since differences between ejaculations are usually large, it was found sufficient to take one sample and count at least 100 sperms, giving a standard error of approximately 7 per cent. As an aid in counting, a hand-tally register was used.

The data obtained from the measurements of volume and density have been analysed under the following headings:

(1) Relation between total volume and total number of spermatozoa.

(2) Seasonal variation.

(3) Individual variation.

(4) Effect of successive matings within a short period.

(5) Relations between number of sperms in ejaculate, and number in various sections of the spermatic tract.

In addition to the above, observations have been made on the sexual behaviour of the male to the live female and to the dummy. It should be noted that the animals studied in these experiments were for the most part rejects from the main breeding colony. As such they probably contained a higher proportion of sterile or partially sterile animals than normal. Representatives of various inbred strains were used. These strains are denoted by letters prefixed to the numbers given to the rabbits where these appear in the text and Tables and are denoted also by separate symbols in Figs. 2, 3 and 4.

### RELATION BETWEEN TOTAL VOLUME AND TOTAL NUMBER OF SPERMATOZOA

The total ejaculate is made up of the sperm, i.e. the dense suspension of spermatozoa in a small quantity of fluid which comes from the ampullae and vasa deferentia, plus the secretions from the accessory sexual glands. If in the process of ejaculation there were always the same

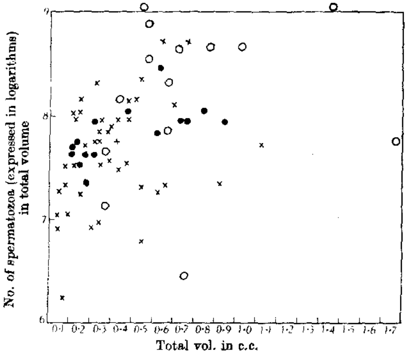


Fig. 2. Chart showing the relation between the total volume of the ejaculate and the number of spermatozoa in the total volume (expressed in logarithms).

F = ●. C = ○. E = ×. S = +.

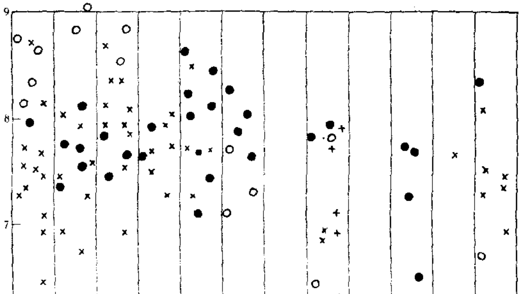


Fig. 3. Variations in the total number of spermatozoa associated with the different months of the year (total no. of spermatozoa expressed in logarithms).

F = ●. C = ○. E = ×. S = +.

proportionate discharge from all parts of the tract, the density of spermatozoa would remain relatively constant and there would be a linear relation between the total volume of the ejaculate and the total number of the spermatozoa.

Text-fig. 2 shows that while there is a general tendency for the number of spermatozoa to increase with the volume of the ejaculate, the relationship is by no means exact. The density of spermatozoa in the ejaculate varies considerably. This may be a feature of importance in considering the potential fertility in different animals. Although in general the probability of fertilization will be greater the larger the number of spermatozoa ejaculated, if this is at the same time accompanied by a relatively greater dilution, the spermatozoa may be handicapped, since experience with semen *in vitro* indicates a better survival in more dense suspensions.

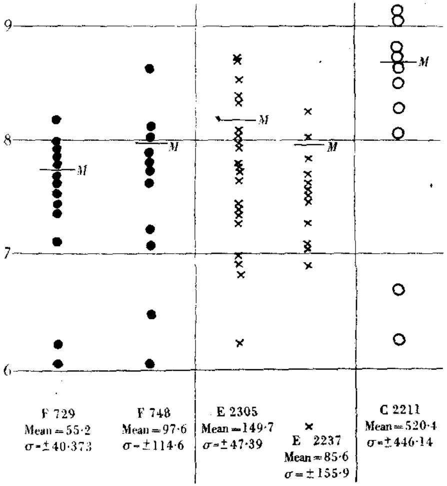


Fig. 4. Chart showing individual variations in total number of spermatozoa (expressed in logarithms).

F = ●. E = ×. C = O.

## Seasonal variation

Text-fig. 3 records the total number of spermatozoa in a series of ejaculations produced by bucks of various strains throughout the year. Previous to the collection, the buck had not been mated within 24 hr. While the results show considerable variation, there appears to be a slight tendency for the numbers to be highest in the early part of the year, and at their lowest during the summer and early autumn. This is in general agreement with the experience of the practical breeder, who finds the fertility of his stock is lowest when the animals are moulting in August and September.

## Individual variation

Text-fig. 4 illustrates samples obtained from five different bucks throughout the year. In each individual, variation is considerable, ranging in one case (C 2211) from just over a million to just under 1000 million. Differences between individuals are probably significant, but as determinations are not corrected for seasonal variation or for variables associated with the technique, probably the difference between individuals is actually greater than that shown. The individual differences shown above may perhaps be related to strain differences, but data sufficient to demonstrate this point are hardly adequate to make this clear.

## Effect of successive matings within a short period

It will be seen from the figures in Table I that, after the second mating, there is a marked falling off, particularly in the volume of the ejaculate. The density, however, remains relatively constant. These results are in general accord with those obtained by Lloyd-Jones & Hays (1918) from samples of semen collected from the vagina of the living rabbit. These workers used, however, specially selected males, and made many more successive matings within a very limited number of hours.

Table I. *Effect of successive matings within 1 day*

| | Ejaculated | | | | | |
|---|---|---|---|---|---|---|
| | 1st | 2nd | 3rd | 4th | 5th | 6th |
| No. of bucks from which determinations made | 9 | 9 | 7 | 6 | 1 | 1 |
| No. of separate determinations | 30 | 30 | 15 | 8 | 1 | 1 |
| Average volume of ejaculate in ml. | 0·32 | 0·28 | 0·14 | 0·12 | 0·04 | 0·10 |
| Average density of sperm in millions per ml. | 200 | 206 | 122 | 227 | 61 | 129 |
| Average total number of sperms in ejaculate expressed in millions | 73 | 41 | 16 | 26 | 2 | 13 |

### RELATION BETWEEN THE NUMBER OF SPERMS IN THE EJACULATE AND THE NUMBER IN THE VARIOUS SECTIONS OF THE SPERMATIC TRACT

The number of sperms in the ejaculate must bear some relationship to the number of sperms available in the male tract. Since presumably the spermatozoa will be drawn first from the ampulla, secondly from the vas deferens or part of it and, thirdly, from the cauda epididymis, a comparison of the number of spermatozoa in the ejaculate with that present in various sections of the tract would indicate the actual sources of supply. For this purpose a number of males were killed by asphyxiation with coal gas. This method of killing is rapid, and does not produce spontaneous ejaculation as occasionally happens when the male is killed by a blow behind the ears. Immediately after death the abdomen was opened, the testis was pushed up from the scrotnm on each side, and the connexion between the testis and the base of the scrotum severed. A ligature was then placed between the end of the cauda and the beginning of the vas deferens. The junction between the ampulla and the urethra was severed and the whole reproductive tract was removed. Each different section was then placed in a receptacle containing a known quantity of saline and cut up into small pieces to liberate the spermatozoa into the surrounding fluid. An estimate of the total number of spermatozoa obtained by this method was then made. The results are shown in Table II.

The animals are arranged in three groups. Gronp A consists of males from which a number of ejaculations had been previously obtained by means of the dummy. It will be seen that the average number in the ejaculate is somewhat larger than the total number of spermatozoa obtainable from the ampullae and vasa deferentia together. This would indicate that, on ejaculation, the ampullae and vasa deferentia are emptied and some spermatozoa drawn from the supply in the cauda. The number iu the ejaculate was always less than the number in the ampullae, vasa deferentia and caudae together, indicating that in an ejaculation spermatozoa are not drawn from the caput epididymis. Gunn (1936) has found that after electrically induced ejaculation in the ram the vas deferens is emptied of spermatozoa and some are drawn from the cauda epididymis.

In Groups B and C are the data of animals which (B) responded to the doe and copulated, but did not respond to the dummy, and (C) responded neither to doe nor to dummy. The object in separating these

Table II. *Spermatozoon content of male tract (in millions)*

Group A. Responded to dummy

| | Ampulla | | | Vas deferens | | | Cauda | | | Caput and body | | | Total | Mean of previous ejaculations | No. of ejaculations |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rt. | Left | | Rt. | Left | | Rt. | Left | | Rt. | Left | | | | |
| E 2305 | 9·6 | 9·6 | 19·2 | 40·0 | 17·2 | 57·2 | 354·0 | 352·0 | 706·0 | 20·8 | 10·0 | 30·8 | 813·2 | 117·1 | 18 |
| E 2237 | 0·8 | 0·9 | 1·7 | 9·6 | 5·8 | 15·4 | 53·5 | 33·1 | 86·6 | 3·5 | 4·0 | 7·5 | 111·2 | 38·82 | 18 |
| E 2471 | 1·5 | 1·4 | 2·9 | 26·2 | 47·4 | 73·6 | 190·4 | 277·4 | 467·8 | 64·0 | 20·4 | 84·4 | 628·7 | 68·0 | 14 |
| E 2489 | 1·0 | 0·2 | 1·2 | 61·7 | 28·0 | 89·7 | 166·4 | 222·4 | 388·8 | 36·3 | 24·7 | 61·0 | 540·7 | 43·075 | 8 |
| F 736 | 9·0 | 8·0 | 17·0 | 82·0 | 20·0 | 102·0 | 200·0 | 200·0 | 400·0 | 24·2 | 38·8 | 63·0 | 582·0 | 153·01 | 10 |
| W 1937 | 5·2 | 8·6 | 13·8 | 182·6 | 112·9 | 295·5 | 366·4 | 202·4 | 568·8 | 220·0 | 112·0 | 332·0 | 1210·1 | 301·9 | 8 |
| W 1956 | 17·0 | 14·0 | 31·0 | 56·8 | 44·9 | 101·7 | 385·4 | 380·0 | 765·4 | 70·4 | 61·1 | 131·5 | 1029·6 | 170·0 | 8 |
| S 2190 | 1·8 | 2·2 | 4·0 | 17·9 | 14·6 | 32·5 | 134·9 | 88·8 | 223·7 | 6·8 | 3·0 | 9·8 | 270·0 | 38·1 | 5 |
| Group A means | | | 11·35 | | | 95·95 | | | 450·887 | | | 90·0 | 523·18 | | |

*Group B. Responded to doe but not to dummy*

| | Ampulla | | | Vas deferens | | | Cauda | | | Caput and body | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rt. | Left | | Rt. | Left | | Rt. | Left | | Rt. | Left | | |
| C 2414 | 0·3 | 0·2 | 0·5 | 64·9 | 62·0 | 126·9 | 424·0 | 242·4 | 666·4 | 11·2 | 17·6 | 28·8 | 822·6 |
| C 2532 | — | ·1 | 0·1 | — | ·2 | 0·2 | 15·6 | 13·5 | 29·1 | 1·2 | 0·1 | 1·3 | 30·7 |
| C 2495 | 1·3 | 0·3 | 1·6 | 5·5 | 9·8 | 15·3 | 170·0 | 215·0 | 385·0 | 42·0 | 3·2 | 45·2 | 447·1 |
| S 2197 | 3·4 | 12·4 | 15·8 | 370·8 | 55·4 | 426·2 | 360·0 | 345·0 | 705·0 | 23·4 | 1·6 | 25·0 | 1172·0 |
| S 2199 | 10·6 | 9·4 | 20·0 | 144·0 | 186·0 | 330·0 | 800·0 | 816·0 | 1616·0 | 4·7 | 4·0 | 8·7 | 1974·7 |
| V 2164 | 3·3 | 3·9 | 7·2 | 126·0 | 124·3 | 250·3 | 718·4 | 606·0 | 1324·4 | 39·6 | 34·2 | 73·8 | 1655·7 |
| Group B means | | | 7·53 | | | 191·483 | | | 787·5 | | | 30·46 | 1017·13 |

Group C. Responded neither to doe nor to dummy

| | Ampulla | | | Vas deferens | | | Cauda | | | Caput and body | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rt. | Left | | Rt. | Left | | Rt. | Left | | Rt. | Left | | |
| C 2143 | 0·6 | 0·2 | 0·8 | 6·6 | — | 6·6 | 100·0 | 118·7 | 218·7 | 9·8 | 4·5 | 14·3 | 240·4 |
| C 2399 | 0·3 | 0·2 | 0·5 | 16·6 | 21·8 | 38·4 | 275·0 | 166·4 | 441·4 | 61·4 | 3·1 | 64·5 | 544·8 |
| C 2409 | 1·0 | 0·7 | 1·7 | 24·7 | 114·0 | 138·7 | 304·9 | 234·8 | 539·7 | 34·4 | 22·4 | 56·8 | 736·9 |
| W 1992 | 9·8 | 24·2 | 34·0 | 40·0 | 62·0 | 102·0 | 285·0 | 220·0 | 505·0 | 55·7 | 216·0 | 271·7 | 912·7 |
| Group C means | | | 9·25 | | | 71·425 | | | 426·2 | | | 101·825 | 608·7 |
| Groups A, B and C means | | | 9·6 | | | 122·34 | | | 557·65 | | | 72·783 | 762·394 |

various groups was to see whether the sexual behaviour of the animals has any direct relation to the number of spermatozoa in the tract. The animals in Group B had more sperm than in either of the other two groups, the difference being most marked in the cauda, but the variation is very considerable and no definite conclusion can be drawn from the small number of data available.

If the number of spermatozoa in the tract is an expression of spermatogenetic activity, it would appear from these figures that there is little direct connexion between spermatogenetic activity and sexual libido. On the other hand, perhaps storage capacity in the tract is not a sufficiently accurate criterion of spermatogenetic activity, since a diminished sperm production may yet be sufficient to maintain the level of spermatozoa stored in the various parts of the tract, while active sperm production would result merely in a more rapid transit.

## OBSERVATIONS ON SEXUAL BEHAVIOUR

No specific experiments on sexual behaviour are described in this paper, but several points of interest in the reaction of the male to the dummy have been observed, and are put on record here as they may be of value to workers specially interested in behaviour.

In common with other animals, the rabbit has a well-marked pattern of sexual behaviour. This pattern can be modified by environment or changes in management. For example, it is well known to practical breeders that much time is saved when making matings if the female is introduced into the cage of the male and not vice versa, because if the male is taken from his cage and placed in unfamiliar surroundings, he pays little attention to the female until he has made a thorough examination of his new environment. This examination consists of smelling round the cage, nibbling at straws or projecting edges of posts, etc., and rubbing the underside of the chin against solid objects such as a food dish or the corner posts and projecting edges of the cage doors. This examination may occupy some time before the male directs any attention to the female. When the female in the strange cage is at last discovered or when a female is introduced into a cage with which the male is already familiar, the male applies to her much the same examination, smelling her, nibbling at her fur and sometimes rubbing his chin on her back. Some males show much excitement, jumping about the cage or to and fro across the doe, mostly emitting a peculiar whine or snore. The male then mounts the female, straddling her with his forefeet and gripping the fur of her back in his teeth. Very rapid pelvic oscillations are combined with

exploratory thrusts of the erect or semi-erect penis. Frequently, more often with young sexually inexperienced males, the buck will mount the female at the side or head. In these cases copulatory movements cease after a time and the male dismounts presumably because the penis has not encountered the vulva or equivalent orifice. Should, however, the buck mount the female at the hind end, the latter, if in oestrus, responds by raising the hind quarters and depressing the back. Lordosis is also further increased when the penis comes in contact with the vulva. Intromission is almost immediately followed by a vigorous copulatory thrust accompanied by ejaculation. The hind legs are thrown forward and the male not infrequently falls over backwards. The ejaculation of semen is accompanied by a very distinct orgasm, and there is no difficulty in determining when it has taken place. The response of ejaculation to intromission is so immediate that in all probability it is a direct reflex initiated by the warmth and pressure of the vagina on the penis.

The sequence of events which constitute the pattern of sexual behaviour can be summarized by listing the various stages in order: (1) exploration; (2) smelling; (3) jumping; (4) chin rubbing; (5) mounting; (6) gripping with the teeth; (7) pelvic oscillations; (8) exploratory movements of erect penis; (9) intromission; (10) orgasm with ejaculation. Now although this sequence of events remains fairly constant in all individuals, the rate and persistency with which the pattern is performed varies very considerably from male to male, and in the same male at different times. For example, if we introduce an oestrous female into the cage of one male, the first four stages do not appear or may be passed through with such rapidity that they may scarcely be observed, and even the rest of the sequence may be extremely rapid. Another male, on the other hand, may pass slowly through all the stages or may proceed only to one particular stage and then desist. Some males, especially if ill or in bad condition, may make no response. With reference to a particular standard, in this case the oestrous female, males can be graded according to their sexual drive into various classes of strong or weak males.

Performance of the sexual pattern, however, depends not only upon the sexual drive of the male but also upon the "suitability" of the object upon which sexual activity is expressed. The artificial vagina and the dummy enable one to vary this suitability. For example, very occasionally a "strong" male has been known to complete the sexual pattern and ejaculate when the hand only of the operator was introduced into the bucks' cage. The minimum of suitability in this case is

therefore little more than something to mount. A less active male might mount the hand without ejaculating, but would complete the act if the artificial vagina were presented. A still less active male will not mate with the artificial vagina alone, but will do so if the hand and forearm are covered with fur.

In our experience, therefore, completion of the sexual pattern depends upon two factors; first, the sexual drive shown by the male, and secondly, the suitability of the sexual object. These two factors are supplementary to each other in causing the completion of the sexual pattern. For example, a strong male requires only the minimum of suitability of the sexual object to produce ejaculation, while a weak male may require the maximum of suitability (i.e. oestrous doe). We have used the term "suitability" of the sexual object in order not to commit ourselves at this stage on the question of whether sexual "attraction" or "stimulation" are useful terms to use in this connexion. It is clear that with a strong male no attraction or specific stimulation is apparent, The sexual pattern is performed in almost complete absence of anything which could be described as attractive or stimulating; the essential factor is here the sexual drive which, when a suitable object is provided, finds its expression in a complete sexual pattern. On the other hand, it must be noted that a weak male is not merely inhibited by lack of suitability of the sexual object, but will in many cases make no attempt to mate with the dummy. It will, however, immediately show considerable activity, and ultimately mate with the oestrous doe, indicating that some characteristic of the normal female does apparently stimulate the male to increased sexual activity.

A more complete analysis of sexual behaviour cannot be given, but it is hoped that these notes may show a new experimental approach to an important aspect of animal behaviour.

## SUMMARY

A method of obtaining samples of semen from rabbit males by means of a dummy and artificial vagina is described. The volume of the semen, and the number of spermatozoa per cm. (density) and the total number of spermatozoa in the sample have been measured. The data obtained from these measurements have been analysed under the following headings:

1. *Relation between total volume and total number of spermatozoa.* With an increase in the total volume of the ejaculate there is an increase in

the total number of spermatozoa, but the relationship is not linear. The density of spermatozoa varies considerably.

2. *Seasonal variation.* Seasonal variation in sperm production is not marked in these data. There is slight tendency for the numbers to be highest in the early part of the year and lowest in August and September when the animal is moulting.

3. *Individual variation.* In each individual, variation is considerable. Differences between individuals are not marked, but as most of the animals were of low average fertility, it is probable that this result is not typical of male rabbits in general.

4. *Effect of successive matings within a short period.* In successive matings the number of spermatozoa diminishes rapidly. As the density is not much affected, the diminution is due to reduction in the volume of the ejaculate.

5. *Relation between the number of spermatozoa in the ejaculate and the number in the various sections of the spermatic tract.* After a series of samples had been obtained, some males were killed and an estimate made of the number of spermatozoa present in various parts of the tract. From the results it is concluded that on ejaculation the vas deferens is practically emptied and that some sperm in the ejaculate come from the cauda epididymis.

6. *Observations on sexual behaviour.* The sexual pattern of the male rabbit is described and the relation between sexual stimulation and sex drive is discussed.

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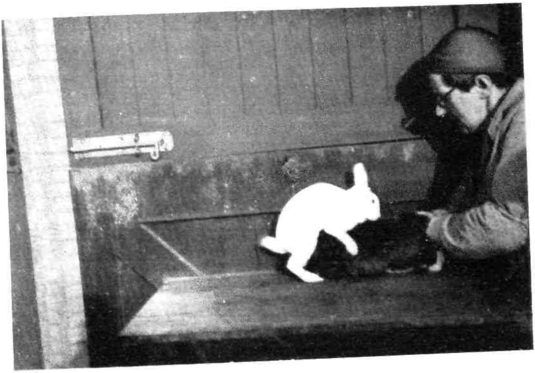

Collecting semen with dummy rabbit.

# OBSERVATIONS ON THE PROTEINS OF PASTURAGE. PHOSPHORUS AND PROTOPLASM

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## INTRODUCTION

CHIBNALL (1924) by his original ether method (1923) prepared "soluble" cytoplasmic protein from the leaves of spinach equal to about 20 % of the total protein and with 16·25 % nitrogen in the dry ash-free substance. Miller & Chibnall (1932) obtaining negligible amounts from various grasses, modified the method by using ether-water instead of ether, and

were then able to make preparations from cocksfoot with 12·3–14 % N (ash-free). "The yield however, varied greatly and in most cases was disappointingly small." After a further modification in which "used" ether-water was employed, Chibnall *et al.* (1933) from growths of pure strains of pasture plants obtained yields ranging from 9·7 to 30·9 %. The nitrogen however "ranged from 10 to 15 % owing to the presence of non-nitrogenous impurities whose chemical composition is not yet clearly understood" (Pollard & Chibnall, 1934).

It is necessary to state that by ether, ether-water, or "used" ether-water, the cells are cytolysed, and vacuole matter washed out by water as the first step. After "used" ether-water the cytoplasmic contents of the cells are stated to be much more readily dispersed into colloidal solution on grinding the material to a pulp with water. Successive extracts from the pulp, each after grinding with water, are combined, filtered, and the proteins flocculated by raising $[H^+]$ up to the isoelectric point with HCl. After siphoning off supernatant liquid, the proteins are coagulated by heating on the water-bath, extracted with boiling water, with ether overnight in a Soxhlet, and by repeatedly boiling with alcohol.

The evidence given in the various publications seems to suggest that whether by ether, ether-water, or "used" ether-water, growths of different species behaved roughly in the same relative manner in regard to the degree of the dispersion and, in general, the lower the relative degree of dispersion the lower the quality of the preparation. On the other hand, preparations from a particular species were of similar quality whatever the degree of dispersion.

In the present paper an account is given of trials of the original ether method and of developments therefrom in the case of pure cultures of perennial rye-grass (P.Rg.) and wild white clover (W.W.C.), and of observations from a general investigation of these cultures on factors probably determining the degree of dispersal of their protoplasmic proteins into aqueous solution. Apparently, with 12·8 and 13·2 as the highest respective percentages of nitrogen (ash-free) mentioned for Chibnall-preparations, these were not amongst the more responsive of the pasture plants. The ether-water modifications have not yet been tried.

The tissues of plants, like those of animals, undoubtedly contain several kinds of protein probably with one kind—the main constituent of the cytoplasm—greatly predominant in amount. From flesh (apart from blood), and even from materials as favourable as fat-extracted seeds, only a moderately satisfactory part of each kind can be separated in a

representative state. Hence, in attempting to ascertain to what extent the various amino-acids could become available to the animal from a foodstuff it has been necessary to follow the plan of determining as closely as possible the relative proportions of the different proteins and, since impurities would be of snch nature as to affect the hydrolysis adversely, to make certain of character and highest quality in order to avoid the waste of the great deal of time, labour and skill required for a satisfactory separation of the various hydrolytic products. The extent to which the different proteins are digested from the foodstuff by the animal then remains to be taken into account. The vegetative tissues of plants, to the greater extent the less succulent, were bound to present preparation-problems of greater difficulty.

Chibnall and colleagues attempted "to prepare grass proteins as readily as possible in amounts sufficient for analysis". Having regard to the special difficulties, further illustrated below, their latest yields seem very satisfactory at the present stage. As they point out, however, there remains the question as to how far the yields of the various amino-acids on hydrolysis may deviate from those true for the pure protein through destructive effects of the impurities responsible for the nitrogen being lower than 16–16·5%. The nature of these impurities is under their investigation. Miller & Chibnall (1932) isolated a polysaccharide from the cocksfoot preparations mentioned (p. 136), leaving, however, the major part of the impurity still unknown. Miller (1935) determined the basic amino-acids in (*a*) a preparation (12·1% N, ash-free) from spring pasturage (chiefly rye-grass), (*b*) the products of hydrolysing what dissolved on boiling the residue obtained from the same pasturage after extracting fatty substances chlorophyll and "all non-protein nitrogenous substances" with 4% HCl for 48 hr. Comparing (*a*) with (*b*) he found the distribution similar in both cases and concluded that "the basic amino-acids probably undergo little if any decomposition during the hydrolysis of a protein in the presence of much carbohydrate material".

## EFFECTS OF CARBOHYDRATES IN PROTEIN HYDROLYSES

Evidence summarized by Plimmer (1917, p. 65) gave rise to the general belief that carbohydrates of various kinds, if present during the hydrolysis of proteins by acids, destroyed several of the amino-acids to some extent through the latter contributing in the formation of humins or melanins. Different amino-acids varied in vulnerability, and different carbohydrates in reactivity. Maillard (1912) obtained melanin-like

substances from alanine and glycine by xylose arabinose and glucose, less rapid in the case of glucose. Roxas (1916) boiled, 48 hr. under a reflux, solutions each 50 ml., containing about 0·1–0·4 g. of certain amino-acids, with about 3–10 times as much fructose or xylose or glucose and HCl at different concentration. The solutions were neutralized with the calculated quantities of soda giving salt which "coagulated most of the precipitate which may have existed in the colloidal state". The humins were filtered off and washed repeatedly with boiling water. The clear filtrates showed different colouring. In the case of 20% HCl he found tryptophane lost 71% of its nitrogen, tyrosine 15, cystine 3·1, arginine 2·3, lysine 2·6, histidine 1·8, whereas alanine, leucine, phenylalanine, and glutamic acid were not factors in the humin formations.[1] The three histone bases reacted with sugars more readily in weak acid or aqueous than in strong acid solutions. In 20% HCl with proline also present, a larger amount of nitrogen disappeared in humin formations from cystine and tyrosine. Fructose and xylose were as a rule more reactive than glucose. Gortner (1916) showed that certain aldehydes, e.g. furfural and formaldehyde, reacted on boiling with HCl in a way similar to carbohydrates, and in the presence of amino-acids the humin nitrogen was large, suggesting the production of furfuraldehydes as an intermediate stage in the formation of the nitrogenous humins. Roxas (1916) also tested the effects of furfural and concluded that its formation from sugars under the influence of acids "may to a great extent be responsible for humin formation".

The following considerations suggest, however, that very little real headway has been made towards an explanation of the events in the production of humins during hydrolyses.

(1) Certain amino-acids, e.g. tryptophane and probably those other than cystine which contain sulphur, are very largely destroyed with the production of humins during hydrolysis by mineral acids even though so far as can be ascertained at present carbohydrates were absent. Williams (1917) (here) obtained very large yields of humins on hydrolysing by mineral acid a protein with very high sulphur, viz. 4·2% (and 15·7% N ash-free), prepared by heat coagulation from the juices of the Swede turnip. It is believed that inorganic sulphur could not account for more than a very minor part and, although sulphide sulphur was yielded on boiling the protein with soda, that the organic sulphur was largely in forms other than cystine.

[1] Indicating that some of the amino-acids and possibly the dibasic amino-acids (other than cystine) as a class may be usefully determined in spite of carbohydrate impurity in the protein. It is noted, however, that Roxas and Maillard differ in regard to alanine.

In view of such inevitable destructions on hydrolysing with mineral acids, rendering necessary the employment of less violent hydrolysing agents in determining particular amino-acids, e.g. trypsin in the case of tryptophane, it seems unfortunate that Roxas, etc. did not include control tests omitting the carbohydrates.

(2) Although the evidence is strongly suggestive, there is no proof that the prior formation of the simplest hydrolytic products from the protein, nor of furfuraldehydes from the carbohydrates are essential in the formation of the nitrogenous humins.

In the Roxas-tests the effects of fructose and xylose were alike, although by the Krober-method these give different types of furfuraldehyde in very widely different yields. The former (or hexoses in general) gives only 1–2 % of the ω-hydroxymethyl variety probably through ready hydrolysis of this variety to formic and laevulinic acids (Cunningham & Dorée, 1914), whereas the latter (or simple pentoses in general), even in 70 of the 120 min. distilling, gives the full quota of ordinary furfuraldehyde, viz. about 51·5 %.

*Experiment.* Part of a stock of hydrolysis-liquor from good casein by 48 hr. boiling with HCl was freed from acid-insoluble humins, and the solution evaporated to a syrup on the water-bath. The syrup was taken up in water and the acid-soluble humins completely flocculated by soda added until the solution was only very faintly acid to congo red paper. The precipitate was well washed with water, redissolved in a small excess of HCl and the dry acid-soluble humins and their nitrogen determined in aliquots. To a quantity of the filtrate plus washings containing 5·53 g. net (after allowing for NaCl) of the "amino-acids mixture", originally in the hydrolysis liquor with 0·168 g. of the acid-soluble humins (7·78 % N), with a total "stages 2 + 3" value of 45·36 ml. N alk. by the alcohol-titration-method (Foreman, 1920) determined in a suitable aliquot, 1 g. of (B.D.H.) xylose was added and the Kröber-method applied. Two controls using 1 g. of the xylose but omitting the amino-acids, in one of which, (B), precautions were taken to avoid all possibility of the charring of solids upon the surface of the glass above the liquid advised by Browne (1912), were conducted in exactly the same manner. In the experiment and controls furfural phloroglucide was estimated in a quarter of the mixed distillates and its xylose equivalent calculated from the Kröber-table.[1] After removing acid-insoluble humins, the residual liquors from the distillations were examined for acid-soluble humins and amino-acids exactly as the hydrolysis liquor.

[1] It is noted that 1 g. xylose is an abnormally high quantity for a Kröber-trial and there was more exposure of the distillates to the air than usual by this mixing.

The total "stages 2+3" values before and after applying the Kröber method were 45·36 and 45·14 ml. N alk.

| Boiled with HCl rising from 12 to about 21% in the course of the Kröber-distillations | From the 1 g. of xylose | | Per 0·25 g. of the xylose used | |
|---|---|---|---|---|
| | Acid-insol. humins | Acid-sol. humins | Furfural phloro-glucide | Xylose by the Kröber-table |
| | g. | g. | g. | g. |
| *Xylose + the "amino-acids mixture"* | 0·0638 | 0·0222 | 0·2497 | 0·2327 |
| *Control. Xylose only* | 0·0366 | 0·0044 | 0·2566 | 0·2390 |
| *Control (B). Xylose only* | 0·0319 | 0·0050 | 0·2570 | 0·2393 |

It is seen that xylose produced very little, indeed if any, destruction although the "amino-acids mixture" included several, e.g. the histone bases, etc., which showed some vulnerability in the Roxas tests, presumably because the furfural through being removed as rapidly as formed never attained high enough concentration. It is noted, however, that fructose, equally with xylose, produced its full effects in the Roxas-tests in spite of its furfuraldehyde being destroyed as rapidly as formed. Hence it is difficult to believe that furfuraldehydes are essential intermediaries in the formation of the nitrogenous humins.

This experiment suggests that adverse effects of any pentosic impurities in protein preparations would be largely avoided by distilling away furfurol as rapidly as formed after the manner of the Kröber-method *but without allowing* HCl *in the residual liquor to exceed say* 12%,[1] and then adjusting the volume and the concentration of HCl for an ordinary hydrolysis under a reflux. Simple pentoses yield their furfural very largely in the early stages of the Kröber-method.

(3) Amino-acids may be conjointly concerned in humin productions. Hence their behaviour when tested singly is not necessarily that which would be exhibited in a hydrolytic mixture.

*Possible relations between the progress of hydrolytic changes and the effects of carbohydrate impurity.* Assuming the prior liberation of amino-acids to be an essential step in the production of nitrogenous humins, it seems inadmissible to infer that a carbohydrate would affect the various amino-acids as they arise during an hydrolysis in the same relative manner as when tested separately. Presumably the reactions would be

[1] *Such a limitation of the* HCl *concentration is not possible by the* Kröber-procedure of replacing successive distillates with HCl of the same strength as that of the original solution. HCl in the residual liquor reached 16·6% and about 20% at the finish of the 1st and 3rd of the twelve 10 min. Kröber-distillations. Constancy of this finish-percentage when removing equal successive distillates is attainable by replacing them with HCl of the strength of the first distillate.

continuously governed by the relative concentration of the reactive components. The composition of the amino-acids mixture varies as the hydrolysis of a protein proceeds in relation to the order in which the amino-acids are split off and their rates of production. On the other hand, if prior changes to the carbohydrates, e.g. into furfuraldehydes probably preceded by hydrolysis if complex, are essential for providing the reactive agents, then the concentration of the latter would vary according to the rates of these changes. Also the relative concentration of the reactive components on hydrolysing a proteiu and a carbohydrate together would depend upon the progress of their elimination in the formation of humins. Hence it appears that the extent to which the yield of a particular amino-acid would deviate from that true for the pnre protein would depend upon the concentration of the reactive carbohydrate agent at the time the amino-acid was split off and, although vulnerable to the destructive effects wheu tested apart, it might even escape altogether if produced after the whole of the carbohydrate had been used up. It follows that the same carbohydrate as the same percentage impurity in two proteins of unlike composition might cause a differing percentage deviation from the true in the yield of some particular amino-acid. Also, it seems probable that the more vulnerable amino-acids of a mixture would tend to protect those less vulnerable.

From these standpoints it appears that Chibnall-preparations with only 25–30 % of non-nitrogenous impnrity in the least satisfactory cases may yet yield practically the right amounts even of some of those amino-acids which are somewhat vulnerable to carbohydrate effects when tested singly. Unfortunately, comparisons of determinations of various hydrolytic products from preparations impure to a differing degree, with no guarantee, however, that the impurity is always the same substance or the same substances in the same proportions, afford practically the only means at present available of judging the effects, and the determinations entail questions as to the merits of the methods employed. It is evident that specimens of uudeniable purity, however poor in yield, would be very valuable at the present stage.

Also, when the quantities of carbohydrates are relatively very large and very undesirable nitrogenous substances are also present (p. 146) as in the case of direct hydrolyses of the whole grass, or its water-insolnble residue, or even of a 4 % HCl extract of the latter, it seems that amino-acid determinations could not be accepted. Csonka (1935) records a large destructiou of histidine and cystine on hydrolysing yeast directly by acids.

The great progress made by Chibnall and colleagues towards the ready preparation of satisfactory proteins from materials so difficult as pasturages is recognized, and the new opportunities arising from their work are appreciated.

### Trials of the original ether method on perennial rye-grass

In agreement with Miller & Chibnall (see p. 135) several trials gave very small yields. One example also illustrates the additional difficulties when dealing with growths past the stage of leaf only:

*500 g. cut 20 June; tops of inflorescences visible; yield about 1% of the total protein.* Percentage nitrogen in the dry solids of:

| Whole grass | Leaf blades | Inflorescences down to the node | Remainder |
|---|---|---|---|
| 2·84 | 4·07 | 3·0 | 1·81 |

### Attempt to obtain improved preparations

Wastage of cytoplasmic proteins during the washing away of the vacuole matter was suspected. An attempt was therefore made to bring them as quickly as possible into a "salted out" state by washing with saturated aqueous ammonium sulphate instead of water so that they should remain intact in an insoluble but reversible form during the washing, which could then be done very thoroughly without fear of continuous loss.

After treating with ether and removing as much vacuole fluid as possible by means of a "tincture press" (p. 148), the material, in its cloth, was allowed to imbibe its full load of saturated aqueous ammonium sulphate and pressed as before. After at least six similar treatments, the third generally giving an almost colourless express, the material was ground with water, of volume equal to the original weight of grass, to a fine pulp in a Nixtamal mill. Three successive extracts using one-third the above volume of water for the last two, crystal clear on filtering through a pad of filter paper pulp, were made. The first contained 6% ammonium sulphate, the second and third progressively much less. In some cases even the first had only a very light amber colour. After diluting the first with an equal volume of water, precipitates were obtained from all three as usual by HCl up to the isoelectric point. The first gave much the largest precipitate, the third very little. The three precipitates, combiued, were washed several times by decantation after stirring and long soaking in large volumes of water. The product was then washed copiously with cold water on an ordinary filter, from which it was detached into a beaker by a jet of rectified spirit. The spirit was gradually

replaced in the course of several days by absolute alcohol, and the latter by anhydrons ether, in the usual way.

From P.Rg. the precipitate was dark grey; from W.W.C. grey-white. Dried in desiccator at R.T.: hard dark grey cakes from P.Rg., and soft light grey cakes, easily reduced to a voluminous soft powder, from W.W.C.

By this process preparations were made from contiguous first cuts of adjoining pure (uniform) cultures of P.Rg. (culture I) and W.W.C. produced from seed sown on 26 April. The P.Rg. eventually produced practically no inflorescences in this first season.

Table I and "Brief notes on the weather" convey sufficient for present purposes in regard to the progress of growth and the main variations in the conditions.

Table I

| | Perennial rye-grass (culture I) | | | | | | | | Wild white clover | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Cuts | 1st | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | B | C | D | E |
| Date ... | 5 June | 8 June | 11 June | 15 June | 18 June | 22 June | 27 June | 30 June | 7 July | 11 July | 18 July | 24 July |
| Cut at ... | 3* | 10·30 | 11·45 | 12·30* | 11·30 | 10·45 | 11 | 11·15 | 11·30 | 11·20 | 11·30 | 11·15 |
| Approx. yield of total dry solids per unit area as % of the eventual maximum | 3·1 | 6·8 | 9·7 | 9·2 | 12·5 | 21·5 | 30·4 | 37·5 | 13 | 16·4 | — | 19·1 |
| Approx. % increase in the yield of T.D.S. above the preceding cut | — | 120 | 44 | Nil | 27 | 72 | 41 | 23 | — | 26 | — | 16·5† |

* Times are a.m., excepting those marked with an asterisk, which are p.m.
† % above C.

*Brief notes on the weather*

*Preceding the 1st cut:* A warm very dry period of 11 days, the last 3 brilliant but with much lower "min. $t^\circ$ on grass" at 30–34°. No apparent increase in the produce in the last third of this period. Ground very dry

Periods between:

1st and 2nd: Very wet, very little sun. Warmer at night. No rain in last 24 hr. Ground very wet

2nd and 3rd: Moderately wet. Poor sun. No rain in last 24 hr. Ground wet

3rd and 4th: Heavy rain 32 hr. before cutting after 2–3 days good sun. The 32 hr. were practically sunless. The rain and dullness caused drop of 13° in the "max. screen $t^\circ$" but a large rise in "min. $t^\circ$ on grass". Ground wet

For the 4 days of this period:

| | | | | |
|---|---|---|---|---|
| Hours of sun | 14·5 | 9·5 | 9·9 | 0·4 |
| Max. screen $t^\circ$ | 64 | 67 | 75 | 64 |
| Min. $t^\circ$ on grass | 36 | 34 | 48 | 56 |

4th and 5th: Good sun but cooler. Ground dry

5th and 6th: Poor sun, still cool. Ground dry

6th and 7th: 0·2 in. rain 18 hr. before cutting, after 3 brilliant warm days. Ground wet

7th and 8th: 0·04 in. rain 18 hr. before cutting, moderate sun. Ground dry

| | |
|---|---|
| Preceding B: | Moderately warm, poor sun. Ground wet on 4 July, dry on 7 July |
| Periods between: | |
| B and C | Warmer. Very good sun, brilliant for 2 days before cutting; no rain, ground dry |
| C and D | No rain, brilliant hot days, low temperature at night. At cutting the ground was very dry, with cracks |
| D and E | Similar to the above. Continued drought |

Remarks on the state of the ground refer to the appearance of the soil at the surface at the time of cutting.

The yields of the preparations as percentages of the dry protein of the water insoluble parts of the cuts with data so far obtained are shown in Table II. These yields are very inferior to those obtained by Chibnall *et al.* (1933) from the "used ether water" modification. It is noted, however, that the process was under the disadvantage of the too sudden effects of ether in comparison with "fresh" or "used" ether-water (see Chibnall, *et al.* 1933, p. 1880). Interest in them lies mostly in the implications of their composition in the problem of pure preparations. Probably an application of the ammonium sulphate device (p. 142) (or similarly with some other salt) after "used ether water" (instead of ether) would give much further illumination.

Table II

| | Perennial rye-grass (culture I) | | | | | | | Wild white clover | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Cuts | 1st | 2nd, 3rd | 4th | 5th | 6th | 7th | 8th | B | C | D | E |
| Yields of preparations: | | | | | | | | | | | |
| Dry | 4·9 | 3·4 | 2·6 | 2·53 | 2·69 | 2·3 | 3·05 | 2·96 | 2·29 | 3·63 | 2·38 |
| Dry ash-free | 4·48 | 3·16 | — | — | — | 2·05 | 2·62 | 2·75 | — | 3·54 | — |
| % N in the dry ash-free | 13·65 | 14·73 | — | — | — | 11·32 | 11·54 | 14·47 | — | 14·02 | — |
| In the dry: | | | | | | | | | | | |
| % ash | 9·75 | 7·19 | — | — | — | 10·91 | 14·03 | 7·19 | — | 2·44 | — |
| Total P* (org. + inorg. P) as % $P_2O_5$ | 5·84 | 6·45 | — | — | — | 7·7 | 7·97 | 4·29 | — | 0·87 | — |
| % $P_2O_5$ in the ash† | 51·42 | 52·89 | — | — | — | 56·78 | 54·24 | 55·31 | — | 32·04 | — |
| P in the ash as % of the total P in the dry | 85·85 | 58·96 | — | — | — | 80·45 | 95·48 | 92·7 | — | 89·86 | — |
| % of the total P lost in the ashing | 14·15 | 41·04 | — | — | — | 19·55 | 4·52 | 7·3 | — | 10·14 | — |

* Neumann-method.

† In order to reconvert completely any traces of meta- or pyrophosphates possibly produced in the ashing into the ortho variety the ashes were previously digested with boiling strong acids as advised by Hillebrand & Lundell (1929). This was done by digesting the ashes with acids exactly as in the first stage of the Neumann-method.

*Notes to Table II*

(1) The ash content varies but, save D, is high generally; much higher than mentioned by Chibnall and colleagues for a few of their "soluble proteins" from pasturages.

(2) Nitrogen in the ash-free is especially high in 2nd, 3rd and especially low in 7th and 8th.

(3) Except D, $P_2O_5$ in the ash is as high as it well could be for the inorganic bases of the ash to be entirely as phosphates and in all cases loss of phosphorus, even as much as 41 % for 2nd, 3rd, occurred during the ashing.

(4) A remarkable fall in the quality of the product at the more advanced growth stage of 7th and 8th. Not so however even for older growths in the case of W.W.C.

*The mineral matter of the preparations*

The ashing was done as gently as possible (Pt dish bottom never more than very faintly red). All behaved as if siliceous in needing prolonged heating before carbon-free. In all cases the ash had a brown-red tinge (iron), greatest for D. The following preliminary data for two must be regarded as rough. The examinations were on much too small a scale.

Ash + HCl evaporated to dry, heated at 150° C. for ½ hr., moistened with HCl, boiled out with water, gave extracts and brown-red residues. The extracts provided the data for Ca, Mg, K by the methods of McCance & Shipp (1933). The residues were largely insoluble when well heated with fusion mixture. The silica was obtained from water extracts of the "fuses" in the usual way.

| | CaO | MgO | $K_2O$ | $P_2O_5$ | $SiO_2$ | Brown-red water-insol. matter in the "fuse" |
|---|---|---|---|---|---|---|
| % in the ash of the protein from: | | | | | | |
| P.Rg. "1st" | 0·94 | 3·6 | — | 49·6 | 7·2 | — |
| W.W.C. "B" | 0·95 | 4·8 | Very small | 54·0 | 2·1(?) | 6·7 |

More thorough examinations proposed.

*Significance of the loss of phosphorus on ignition*

From Note (3) in "Notes to Table II" it is obvious that the preparations contained organic compounds of phosphorus and that these might have been present to a greater extent than the loss of phosphorus on ignition represents if inorganic bases in the ash-phosphates were in the preparations as salts of phosphorus-free combustible radicles.

Since the protein precipitates were exhaustively extracted with alcohol and ether which would remove lecithin and allied substances, it appears that the organic phosphorus may have been in the form of nucleic acid or mononucleotides. Presumably the former could come from α-nucleoproteins in the nuclei and the latter from β-nucleoproteins in the cytoplasm. (According to Jones (1914, pp. 8 and 3‿) the latter do

not occur in nuclei.) If present with proteins in solutions they would precipitate in nucleoprotein forms on adding HCl up to the isoelectric point and thus find their way into the preparations.

Probably the 6% ammonium sulphate in the extraction of the pulp (p. 142), like aqueous salts in general, e.g. 10% NaCl (see below), introduced them by special solvent property, whereas cold water extractions of the pulp would be unlikely to contain them. Hence they are not necessarily in Chibnall-preparations of the water-soluble proteins.

Nucleic acid and, say, guanylic acid as a likely mononucleotide, with 16·14 and 19·3% nitrogen would raise the nitrogen and camouflage corresponding non-nitrogenous impurity in protein preparations, and the bases arising on hydrolysis would behave so as to confuse determinations of histone bases.

It is noted that direct hydrolyses of the whole grass or of whole yeast (p. 141) would contain the whole of these as well as several other similarly undesired bases, e.g. choline from lecithins, etc. The mother substances usually hydrolyse readily. Hence if any are present in extraction residues direct hydrolyses, even of extracts made by boiling these residues with 4% HCl, would be correspondingly prejudiced.

Apparently nucleic acid or mononucleotides might be avoided by heat-coagulating the proteins of pulp-extracts in the presence of a suitable salt at some favourable *p*H instead of precipitating by HCl. Without the salt, however, only mononucleotides would be excluded. Or, on the lines of Clarke & Schryver (1917) for preparing nucleic acid from wheat embryos, by actually extracting them from protein preparations after boiling with alcohol, by digesting with 10% NaCl at 60–80° C.

Comparing the losses of phosphorus on ignition with the growth conditions indicated on p. 143 suggests that the protoplasm was richer in these organic phosphorus substances when, as in the case of "2nd", rapid increase of herbage after recent rain followed a period of warm dry sunny weather during which, through lack of water, growth had apparently stopped. Combining the protein precipitates of "2nd" and "3rd" is regretted.

*Source of the phosphorus in the preparations*

Ether entirely prevents bacterial activity in "exudates".[1] Wholly or even partly saturated with ether they keep "sweet" permanently. Slight very dark deposits usually form gradually in ethery exudates on standing a few days. From P.Rg. such deposits (0·15–0·16 g. per 100 ml.)

[1] Liquors obtained on applying pressure to ether-treated herbages.

had about 20% ash and $P_2O_5$ equal to, at the most, 0·1–0·2 mg. per 100 ml.

The clear liquors of "old exudates" gave dark precipitates on adding enough salts, e.g. sodium chloride, ammonium sulphate, etc. (Fresh exudates not yet tested.) In the case of P.Rg. "8th" of Table I and ammonium sulphate, precipitation occurred between 40 and 70% saturation. Precipitates at 100% saturation, thoroughly washed with an aqueous solution 100% saturated, were easily and entirely soluble in water. Per 100 ml. of exudate these solutions contained 0·456 g. of dry substance with 9·8 mg. of ash and yielded, on adding HCl up to the iso-electric point, 0·103 g. of dry with 4·3% nitrogen and no ash. The washed ammonium sulphate precipitates were entirely devoid of phosphorus.

The yields of alcohol-insoluble phloroglucide, viz. that of ordinary furfural, from fresh P.Rg. exudates by the Kröber-method were only 1–3 mg. per g. of the T.D.S. of the grass on the basis of sap-volume determinations per unit weight described later (p. 148). The pentose of nucleic acid and mononucleotides gives ordinary furfural. Hence even assuming these substances to have been entirely responsible for the furfural from the exudates and that ammonium sulphate would have precipitated them, it does not seem possible for the remainder of the saps in the grass when it was first treated with saturated ammonium sulphate (see p. 142) to have supplied them appreciably to the preparations.

With the above evidence from the "old exudates" in support, e.g. phosphorus-free ammonium sulphate precipitates, etc., there can be little doubt that none of the phosphorus in the preparations inorganic or organic was derived from the remains of the saps.

*Possible difference between the solvent action of 6% ammonium sulphate and water upon protoplasmic matter freed from the cells in the pulping process.*

In regard to (1), (2) and (4) of "Notes to Table II": Bearing in mind that there was hardly any room for inorganic acid radicles other than phosphates in the ashes (see (3) of "Notes to Table II"), and that the extractions with saturated ammonium sulphate before the pulping were very thorough, a comparison of nitrogen and ash in the case of "7th" and "8th" with "1st" suggests that the dilute ammonium sulphate solution with which the pulps were extracted (6% in the first extract, see p. 142) dissolved, in addition to what would have dissolved if plain water instead of 6% ammonium sulphate had been used as in the Chibnall-method, from protoplasmic matter freed from the cells in the pulping (very small

in amount because ether and not ether-water was used, see Chibnall *et al.* 1933, p. 1880), mineralized matter as well as further protein possibly originally in conjugation as water-insoluble matter and, in the later stages or different conditions of growth of "7th" or "8th", these "greater complexes" contained much more non-nitrogenous organic matter. Whatever the nature of the non-protein components they were precipitated perhaps after reconjugating with the proteins on adding HCl up to the isoelectric point.

In this connexion ash-data given by Miller & Chibnall (1932, pp. 397 and 394) for cocksfoot preparations from a single cut by "fresh" ether-water seem significant.

5·1–1·3 % in several specimens of the "soluble" protein.

From their "green colloidal solution" in one of these cases:

7·7 % (and 11·4 % N) in the "whole" protein obtained directly on coagulation by heat.

2·7 % (and 13·7 % N) in the "soluble" protein, precipitated by HCl from the filtrate on filtering off the green matter from a part.

Yield ratio of "whole" to "soluble" = 55·6 : 25.

In this case the major part of the total protein of the green liquor seems to have been in a water-insoluble "greater complex" whose ash was higher than 7·7 % and the minor part either was never in any kind of "greater complex" or had become disconnected from mineralized components on leaving the cells when the sap-free grass was ground with water.

Apparently it would follow that the addition of ammonium sulphate to such green liquors might give a higher yield of soluble protein but, however, with higher ash and perhaps lower nitrogen in the ash-free.

## THE SAP-VOLUME METHOD FOR DETERMINING WHAT MAY BE EXTRACTED FROM THE PROTOPLASM OF PASTURAGES

It is necessary, first, to refer to methods evolved for ascertaining to what extent the structural parts of pasturages give water-extractives not originally in the internal fluids or "saps".

### "*Sap-volume method*"

Treated with ether and pressed as tightly as possible in an ordinary "tincture press"[1] the P.Rg. cuts of Table I which consisted entirely of leaf, "surface-dry" gave volumes of exudates, higher the lower the percentage of T.D.S. in the grass, ranging from 62 to 39 % of the weight of grass or from 72 to 48 % of the total volumes of the respective internal

[1] Gallenkamp Cat. No. 796, fitted with a much thicker base plate.

fluids determined as indicated below. In the following seasons, cuts of more advanced P.Rg., containing growing stem, gave considerably less exudate especially after periods of dry hot sunny weather. Even also in this latter case, however, it is shown that certain water soluble constituents may be quantitatively accounted for, practically, on the assumption that the whole of the water in the cut forms a uniform solution of these constituents at the same concentration as in the exudate. In other words (at any rate after the effects of the narcotic) the composition of the exudates may be regarded for practical purposes as reliably representing the internal fluids as a whole. The evidence, discussion and description of methods by which the volume of this solution per unit weight of grass or of its T.D.S. may be determined will be given in another paper. The figure enables the composition of the "saps" and of soluble and insoluble fractions obtained on extracting the growths in various ways to be compared on the unit weight basis and it is then seen how far the extractions remove substances not originally in the saps. Already it appears that extracts of a kind commonly made in investigations may be very misleading as to the true state of affairs in the natural plants (see p. 162).

Examples of the usefulness of this plan in the case of nitrogen and phosphorus in the saps, and in solutions from exhaustively extracting with hot and cold water, the fresh grass in its natural form and the powdered T.D.S. dried at 85–90° C. are given later.

*Method of extracting the fresh unpulverized growths*

After thoroughly mixing the grasses of the cut 50–70 g. portions are weighed out at the same time as other portions for T.D.S. exudates, etc. Since loss of water occurs on exposure delay after the mixing between the weighings and before all treatments should be avoided.

The portions enclosed in a wrap of good linen are completely immersed in ether for 3 min. and as much as possible squeezed out by torsion applied by the hands. Then as follows:

*Hot water extraction*; "*e. and b.w. treatment*". The flame under a 550 ml. beaker of the tall type two-thirds full of vigorously boiling water is turned off and, immediately afterwards, the linen "bag", partly untwisted, plunged well in and the part containing the grass pushed well down by a stout rod. Remaining ether evaporates instantly. After a few seconds the burner is again lighted, the linen supported just clear of the bottom of the beaker by pulling over the rim enough of the top of the opened bag, and the liquor again brought quickly to active boiling which is continued for 2–3 min. while stray pieces of grass are pressed

under by the rod. As much liquor as possible is then torsioned out and the bag opened to receive from a tap several good washings of distilled water each followed by the torsioning. After three more similar treatments of 2–3 min. boiling, in each case followed by the torsion washings, the material (termed "e. and b.w. insol.") is soaked in distilled water over night, torsion-washed, dried and weighed.

By this treatment the proteins of the protoplasm are heat-coagulated as quickly as possible after the ether effects. The squeeze-liquor obtained after the first boiling shows a slight turbidity due to a fine suspension of coagulated protein which diluted exudates when boiled rapidly usually give, and two torsion-washings are usually necessary before clarity is reached. Undoubtedly vacuole-fluid constituents of all kinds are completely removed.

*Cold water extraction*; *"e. and c.w. treatment"*. The bag containing the squeezed ether-treated material is untwisted under a large volume of cold distilled water in which it is torsioned and untwisted several times and then torsion-washed as above. After soaking in fresh distilled water this whole procedure is repeated several times on the first day and twice a day, viz. after soaking over night and throughout the day, for several subsequent days. Finally (termed "e. and c.w. insol.") it is dried and weighed.

*The extent to which "e. and b.w. treatments" dissolve from P.Rg. in its natural state nitrogenous and phosphorous substances in excess of those in the saps determined by the aid of the sap-volume method of p.* 148.

The following examples entailing dissimilar circumstances of growth are dealt with in Table IV.

It is always necessary to convey broad ideas of the stages of development and the weather conditions. As a general plan, enough for present purposes is conveyed by such information as that below and in cols. (1), (2), (3), (6) of Table III. For growth free from soil and dead matter and not too far advanced, the dryer the weather the higher cols. (3) and (7) and the lower col. (6). Cols. (3) and (6) for the moistest but "surface dry" cuts of P.Rg. are about 17 and 5 respectively.[1]

It is seen that between this and the other extreme of very severe drought (e.g. cut O) the figures of col. (6) range between about 5 and 2. Similarly as a general plan, cols. (4) and (5) are included to give broad views of the total relative amounts of nitrogenous and phosphorous substances involved.[1]

[1] All columns mentioned in these two paragraphs refer to Table III.

## Table III

| Cuts | Date of cut (1) | Approx. cwt. T.D.S. per acre (2) | % T.D.S. (3) | % in T.D.S.: N (4) | % in T.D.S.: "Total $P_2O_5$" (5) | ml. of saps per g. of T.D.S. (p. 149) (6) | In 100 ml. of saps: Dry solids g. (7) | In 100 ml. of saps: mg. N (8) | In 100 ml. of saps: mg. "Total $P_2O_5$" (9) | In saps per g. of T.D.S.: mg. N (10) | In saps per g. of T.D.S.: mg. "Total $P_2O_5$" (11) | % $P_2O_5$ in ash of saps (12) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A 1st | 5 June | 1·2 | 21·3 | 3·35 | 1·11 | 3·96 | 10·93 | 105·4 | 125·4 | 4·2 | 5·0 | 6·8 |
| B 2nd | 8 June | 2·6 | 17·5 | 3·65 | 1·10 | 4·85 | 6·88 | 62·3 | 102·5 | 3·0 | 5·0 | 7·7 |
| C 3rd | 11 June | 3·7 | 18·2 | 3·39 | 1·18 | 4·64 | 7·41 | 74·1 | 132·1 | 3·4 | 6·1 | 8·9 |
| D 4th | 15 June | 3·44 | 17·8 | 3·47 | 1·30 | 4·79 | 7·36 | 69·1 | 167·4 | 3·3 | 8·0 | 10·3 |
| F 6th | 22 June | 8·1 | 16·9 | 2·98 | 1·17 | 5·04 | 6·31 | 51·7 | 129·5 | 2·6 | 6·5 | 9·2 |
| H 8th | 30 June | 14·0 | 20·6 | 2·43 | 0·97 | 4·05 | 8·54 | 50·3 | 130·9 | 2·3 | 5·3 | 7·4 |
| I | 7 July | 14·5 | 20·0 | 2·23 | 1·03 | 4·22 | 9·16 | 54·1 | 139·4 | 2·3 | 5·9 | 7·8 |
| J | 15 Aug. | 9·1 | 20·3 | 2·36 | 1·29 | 4·03 | 6·57 | 65·3 | 167·9 | 2·6 | 6·8 | 9·5 |
| K | 24 Aug. | 4·7 | 20·4 | 2·77 | 1·41 | 4·03 | 7·18 | 77·9 | 230·1 | 3·1 | 9·3 | 11·3 |
| L | 7 Sept. | 2·9 | 23·2 | 2·27 | 1·44 | 3·46 | 8·32 | 70·7 | 235·3 | 2·5 | 8·2 | 10·3 |
| M | 28 Sept. | 3·0 | 28·0 | 2·68 | 1·47 | 2·74 | 10·30 | 110·9 | 231·1 | 3·0 | 6·3 | 9·3 |
| N | 16 May | 22·8 | 22·8 | 1·70 | 0·91 | 3·6 | 9·71 | 45·8 | 151·9 | 1·7 | 5·5 | — |
| O | 5 July | 14·1 | 36·3 | 1·64 | 1·02 | 1·94 | 13·38 | 99·0 | 291·2 | 1·9 | 5·7 | — |
| P | 2 June | 18 | 23·3 | 1·27 | 0·84 | 3·43 | 7·27 | 49·3 | 145·5 | 1·7 | 5·0 | — |
| Q | 20 Nov. | — | 21·1 | 4·83 | 1·47 | 3·87 | 7·06 | 171·0 | 206·4 | 6·6 | 8·0 | 9·2 |

In all cases the terms "saps" or "exudates" refer to fresh exudates unless stated.

Other aspects of varying composition in these and other saps will be dealt with in further papers.

Culture I in its 1st year:

| | |
|---|---|
| A–H | The first growths of Table I. |
| I | 32 days' second growth |
| J | 39 days' third growth |
| K | 21 days' third growth |
| L | 28 days' third growth |
| M | 35 days' fourth growth. |

Culture I, 3rd year. Cuts from an area dressed in the previous season[1] thrice successively with ammonium sulphate each at 2 cwt. per acre:

N First growth.

O 50 days' second growth.

Culture I, 5th year:

P First growth.

Culture II:

Q A 3rd growth, all leaf 2–4 in., cut at 3 p.m. on 20 November after 5 days with no rain and dry atmosphere. The 2nd and 3rd of these days each had 6–7 hrs. sunshine, the last two none. "Max. screen $t^0$" for the 5 days was 54–57 and "min. $t^0$ on grass" 35–40.

## *Notes to Table III*

(1) Cols. (7), (8) and (9), with the remarks on p. 150, show that sap concentration varies widely with varying weather.

(2) Cols. such as (10) and (11) eliminate differences due to differing water content in the cuts and enable the relative quantities of sap and non-sap constituents in different cuts to be compared on the same basis. (If desired quantities in saps relative to unit weight of protoplasmic proteins at say 16% N, instead of unit weight of T.D.S., can be arrived at by the aid of data in Table IV.)

(3) The concentration of phosphates in the saps (col. (9)) and sap phosphates per g. T.D.S. (col. (11)), probably in spite of good uptake of phosphates into the saps from the soil in cases such as B–H when the soil was favourably moist, were lower the higher the yield of produce and hence of protoplasmic proteins, per unit area.

(4) Wide variations are seen in the percentages of phosphate in sap minerals (col. (12)). Probably percentages of other essential mineral radicles, e.g. K, Na, Ca, Mg, etc. would also have shown variations (see p. 170).

[1] Whether ammonium sulphate would have any sort of effects after so long and with winter intervening is not known.

Table IV. *Percentages of the respective totals in the whole grass, same cuts as Table III*

| Cuts | | Nitrogen: % of the total N in "e. and b.w. insol." (1) | Nitrogen: % of the total N in Saps (2) | Nitrogen: % of the total N in Sol. in "e. and b.w. tr." not in saps (3) | (3) as % of (1) + (3), i.e. as % of protoplasmic N (4) | Phosphorus: In "e. and b.w. insol.": mg. "total $P_2O_5$" per g. of protein (5) | Phosphorus: % of the total P in "e. and b.w. insol." (6) | Phosphorus: % of the total P in Saps (7) | Phosphorus: % of the total P in Sol. in "e. and b.w. tr." not in saps (8) | (8) as % of (6) + (8), i.e. as % of protoplasmic P (9) |
|---|---|---|---|---|---|---|---|---|---|---|
| A | 1st | 87·52 | 12·47 | ·01 | ·01 | 17·4 | 28·7 | 44·7 | 26·6 | 48·1 |
| B | 2nd | — | — | — | — | — | — | — | — | — |
| C | 3rd | 87·83 | 10·15 | 2·02 | 2·2 | 19·2 | 30·2 | 51·8 | 18·0 | 37·3 |
| D | 4th | 85·72 | 9·55 | 4·73 | 5·2 | 18·8 | 26·9 | 61·8 | 11·4 | 29·8 |
| E | 5th | 87·69 | 9·36 | 2·95 | 3·3 | 17·6 | 27·9 | 53·2 | 18·9 | 40·4 |
| F | 6th | 89·02 | 8·73 | 2·25 | 2·5 | 19·2 | 27·2 | 55·8 | 17·1 | 38·6 |
| G | 7th | 83·99 | 7·91 | 8·1 | 8·8 | 23·1 | 31·6 | 61·0 | 7·4 | 19·0 |
| H | 8th | 85·1 | 9·56 | 5·34 | 5·9 | 19·3 | 25·6 | 54·6 | 19·8 | 43·6 |
| I | | 86·86 | 10·24 | 2·9 | 3·2 | 23·0 | 26·8 | 57·0 | 16·2 | 37·7 |
| J | | 81·66 | 11·14 | 7·2 | 8·1 | 23·7 | 21·4 | 52·5 | 26·1 | 54·9 |
| K | | 84·38 | 11·34 | 4·28 | 4·8 | 19·1 | 19·1 | 65·7 | 15·2 | 44·3 |
| L | | 86·59 | 10·78 | 2·63 | 3·0 | 23·8 | 19·4 | 55·6 | 25·0 | 56·3 |
| M | | 79·63 | 11·36 | 9·01 | 10·2 | 26·4 | 24·5 | 44·2 | 31·3 | 56·1 |
| N | | 83·0 | 9·72 | 6·68 | 7·4 | 33·0 | 32·3 | 60·2 | 7·5 | 18·8 |
| O | | 70·2 | 11·71 | 18·09 | 20·5 | 24·8 | 17·5 | 61·0 | 20·9 | 54·4 |
| P | | 84·73 | 13·34 | 1·93 | 2·2 | 41·6 | 33·4 | 59·7 | 6·9 | 17·1 |
| Q | | 85·32 | 13·78 | 0·9 | 1·0 | 18·9 | 33·0 | 55·0 | 12·0 | 26·7 |
| The "($AS_2$)" Oct. 5 growths of Table VI: | | | | | | | | | | |
| The 3 cwt. | | 76·38 | 11·16 | 12·46 | 14·0 | 17·9 | 22·6 | 48·6 | 28·8 | 56·0 |
| The 6 cwt. | | 74·55 | 13·11 | 12·34 | 14·2 | 19·5 | 29·5 | 44·1 | 20·4 | 47·2 |

Supported by Tables V and VI it is evident that considerable variations in the relative amounts of constituents in the saps probably related so far as the mineral radicles are concerned with what is presented[1] to the plant in the soil water and large differences in concentration merely due to loss or gain of water may occur in varying circumstances of growth.

### *Notes to Table IV*

(1) Col. (4) varying widely, suggests the degree to which wastage of protoplasmic proteins may occur in the washing out of vacuole matter for protein preparation purposes.

(2) *Broadly for A–H, the higher col.* (9) *and the lower col.* (4), *viz. the higher the percentage of the protoplasmic phosphorus in water-soluble forms and the lower the capacity of protoplasmic heat-coagula to yield nitrogen in water-soluble forms the higher the protein yields of Table II.*

(Applications of these methods of investigation side-by-side with protein preparations via "used ether water" instead of ether (see pp. 136, 147) are now needed.)

(3) *As an outstanding feature, apparently for second or further successive growths of the season, cut late enough in the season, col.* (4) *may increase greatly with increasing severity of drought.*

### *Discussion arising from Tables III and IV*

(Columns mentioned without stating their table refer to Table IV)

Changes in the relative quantities and concentration of the constituents in the vacuole fluids, entailing related changes in their buffering capacity and perhaps in their *p*H, would be expected to influence building or breakdown of proteins in the protoplasm.

Col. (3) or (4) shows the extent to which protoplasmic nitrogen was present in water-soluble forms not coagulable in boiling aqueous solutions. Apparently, at any rate in the case of young leaf growths with dilute saps (see later), since still in the protoplasm, these substances were either the more complex intermediaries in the protein building perhaps such as albumoses, or the more complex hydrolytic derivatives in protein breakdown.

Col. (3) is especially low for A, practically at the seedling stage, and Q, the cut of 20 November at the stage of 2–4 in. leaves. In A, growth had been stopped for some time through lack of water and in Q, as usual in late November, it had been for some time either stopped entirely or extremely slow. Hence, assuming col. (3) to be due to uncompleted building, it would follow that the less recent or the less rapid the building of protoplasmic proteins the smaller their content of the intermediates.

[1] *Undoubtedly during the course of the season the relative amounts of various mineral radicles in the soil water vary with the varying conditions in the soil accompanying changing weather. Thus considerable change in these ratios would be expected to accompany the drying out of the soil in drought.*

Col. (3) is considerable and varying in the young moist leaf growths B–H and these values seem to reflect, either the recency of formation suggested above (not supported however for D, more closely examined below), or breakdown following some kind of change-over in the conditions common to all. If the latter it would appear that, continually during active leaf growth, building and breakdown of a part of the protoplasmic proteins may alternate with alternating conditions of temperature, sunlight, dilution, composition, buffering capacity and perhaps *p*H, etc., of the saps, such as accompany the change-over from day to night.

Assuming col. (3) to reflect breakdown only, the data in Tables III and IV seem to afford illustrations of the following situations in regard to the building or breakdown of protoplasmic proteins:

(1) Cut A. Building stopped some time before cutting through the effects of very dry weather and no breakdowu in the interval. Highly concentrated saps, very rich in "nitrogen" (see cols. 7 and 10 of Table III).

(2) B–H. Breakdown in young leaf growths under no lack of water probably as the alternate phase to very recent building. Dilute saps mostly rich in "nitrogen".

(3) Q. Alternate building and breakdown in a young leaf growth brought practically to a standstill by cutting time under the unfavourable conditions of temperature, sunlight etc. of late November. Dilute saps extremely rich in "nitrogen".

(4) M and O. Building stopped sometime before cutting by drought. High and very high degrees of breakdown during the further extension of drought. High and extremely high concentration of saps. Breakdown products retained by the protoplasm.

(5) N and P. Tall first growths of the seasons, at the stages of leaf increase nearly and entirely stopped, which at any rate for some time before cutting, and more so for P, had been starved for "nitrogen" by a soil much exhausted by removal of previous cuts. P, the more advanced, had dilute saps, more dilute than N, although the growths had about the same percentage of water. The saps were both very poor in nitrogen per g. of T.D.S.: breakdown of protoplasmic protein followed by discharge of products into dilute enough saps and utilization of those nitrogenous in developing the inflorescence.

The richer the produce in protoplasmic proteins the richer the saps in "nitrogen" per g. of T.D.S. The saps of A and M were very rich, and rich in "nitrogen" and, apparently, building rather than breakdown of proto-

plasmic protein had been favoured right up to the stoppage of growth by very dry weather in A and by moderate drought in M. In both cases the protoplasm was very rich in water-soluble phosphorous substances (col. 8), and very poor in the percentage of the total phosphorus found in the saps (col. 7). Hence it appeared that phosphates had been withdrawn by the protoplasm from the saps.

In all cases save O, either col. (8) is high when col. (7) is low or col. (8) is low when col. (7) is high.

*Drought effects.* Loss of water as one of the effects of drought concentrated the saps very remarkably (cf. in Table III: F, the moistest; M and O, moderate and very severe drought). Probably sap concentration is of great importance in determining building or breakdown processes. Apparently, the rate of growth of grass and hence of protoplasmic proteins, etc. progressively falls to zero as the general sap concentration rises to a high enough point and, with further rise beyond this point, perhaps provided the temperature is sufficiently high and the less rich the saps in amino substances, breakdown by natural proteolytic agents progressively develops. This is indicated in Table IV by low in col. (1) and high in col. (3) moderately for M and greatly for O. It seems likely that in the case of O breakdown may have advanced further than the more complex protein derivatives.

Cols. (8) and (3) are both high for M and O. Hence it appears that the breakdown changes fell on that part of the protoplasmic proteins originally "associated" with water-soluble phosphates and that the breakdown products from this "greater complex" (hereafter called the "easily resolved greater complex" or "Complex *a*"), viz. protein derivatives and the freed phosphate component, owing to the high concentration of phosphates and other constituents in the saps, were retained by the protoplasm. Doubtless, however, the products would have entered the saps if these had been diluted enough by water taken up after rain. In the case of breakdown in growths with dilute saps col. (8) is low and col. (7) high. Hence the freed phosphate component at any rate, and probably the whole of the protein derivatives eventually, are excreted into dilute enough saps. In the case of O the breakdown seems to have gone on far enough even for the protoplasm to have lost some of the freed phosphate component to very strong saps.

Col. (8) is high, col. (3) extremely low and col. (7) low for A. Cols. (3), (6) and (7) of Table III, with the remarks on p. 150, show that the very dry warm weather preceding the cutting of A (A and "1st" of Table I are the same cut, see weather notes on p. 143) could hardly be called drought

and that growth processes had been stopped through the saps, because very rich in nitrogenous and other metabolites, readily attaining a high enough concentration. Apparently the dry situation had not been sufficiently prolonged nor severe enough for the breakdown such as occurred in M and O to begin. Hence, the protoplasm of A was rich in water-soluble phosphates and therefore rich in "Complex *a*" which had remained intact and phosphates had been withdrawn from the saps for the building of phosphate-protein-complexes whilst the saps were taking up little or none from a dry soil.

D showed no increase over C neither in produce (see 3rd and 4th = C and D, in Table I) nor (determined) in protoplasmic proteins. Col. (3) shows considerable protein breakdown in the protoplasm. In the period between C and D the conditions before and after the heavy rain changed from warm and sunny by day and very cold "on the grass" at night to much cooler and sunless by day and very much warmer "on the grass" at night (see weather notes on p. 143). Apparently the conditions after the rain were unfavourable to the building and favourable to the breakdown, and if there had been any building in the former part of the period it had been compensated by breakdown in the latter part. Comparing D with A, col. (8) is much lower (much less "Complex *a*"), col. (3) shows considerable breakdown for D but none for A, and col. (7) is very much higher for D probably due partly to discharge of phosphates from the breakdown of "Complex *a*" into dilute saps and partly to the accumulation in the saps of phosphates taken up from the favourably moist and (at this time) fertile soil during the period between C and D when there was no building of protein-phosphate-complexes and therefore no demand for phosphates by the protoplasm from the saps.

Q resembles D in stage (both "all leaf", 2–4 in.) moistness of soil, general concentration of saps (col. (7), Table III), almost similar corresponding values in Table IV save col. (3) showing breakdown very small for Q and much less than for D, similar type of unfavourable weather for growth for the last $1\frac{1}{2}$–2 days in each case after 2–3 days with sun; differing however in the day temperature being lower and the "min. $t^0$ on grass" much lower for several days prior to the cutting of Q (cf. weather notes on pp. 143 and 152). Per g. of T.D.S. the saps of Q were rich[1] in phosphates (equal to D) and extremely rich[1] in "nitrogen" (twice the good supplies in D). The produce was extremely rich in protoplasmic proteins (richer than D). Hence it appeared that when the

[1] The saps of very long much older leaves from another P.Rg. culture cut at the end of October were very similarly rich.

conditions of temperature, sunshine, etc., or the content of various essentials in relation to their effects, were unfavourable to building in spite of apparently good supplies of nitrogenous materials and phosphates in the saps, they were yet somewhat favourable to breakdown in the case of the protoplasmic proteins of D, but with the much lower temperatures characteristic for November in the case of Q these conditions became very unfavourable for breakdown as well as building. Apparently in Q for many days there may have been a very little alternate building and breakdown with the collective balances in favour of a very small net production enough to render an extremely small growth of the grass very rich in protoplasmic proteins. Possibly, however, this richness may have been due to a smaller production of "thickeners" accompanying poor carbohydrate production in the adverse circumstances of November. Also since col. (8) is low very recent protein breakdown may have been greater than col. (3) indicates owing to discharge of the products into dilute saps and the excessive richness of the saps in "nitrogen" may have been due largely to the accumulation of such products from the protoplasm. The significance of other features in the composition of these saps is also under study. A continuance of the examinations throughout the winter is needed.

Col. (5) ("total $P_2O_5$" per g. of protein in the "e. and b.w. insol.") varying widely, is higher the more advanced the growth, the lower col. (8) and, except after drought when the protoplasm retained the breakdown products (p. 156), the higher col. (7). Thus when the saps were dilute enough col. (5) was higher the less rich the protoplasm in "Complex *a*", presumably after some had been broken down to yield its products to the saps. These features are very prominent in cuts N and P which had advanced as far as inflorescences half and fully out of sheaths respectively.

For old enough growths of P.Rg. the percentage of leaf blade[1] solids in total solids progressively decreases with increasing advancement of growth, but for such growths at the same stage varies widely with varying soil fertility probably with "nitrogen" supply from the soil as the chief limiting factor. For P, it was only 13·1 and nitrogen in the blade solids only 2·13 %. For a more luxuriant growth on another soil nearly as far advanced (tops of inflorescences visible) the figures were 36·5 and 4·07 %.

For N and P nitrogen in saps per g. of T.D.S. was very low (Table III). Evidently they had been very poorly supplied with "nitrogen" by a much exhausted soil (see Note (5) on p. 155) and protein building in

[1] Cut off at the sheath-tops.

leaf protoplasm had been at a very low level. Hence, apparently, to a greater extent than in better growths at these stages, they had to depend upon leaf protoplasm and upon much smaller amounts for protein breakdown products for developing the inflorescence, with the result seen in the extreme lowness in col. (8) that the protein-containing-complexes had been very largely exhausted of "Complex *a*". Probably the highness in col. (7) was due partly to discharge of the freed phosphorous component and partly to the phosphate supply from the soil having been in excess of the limiting nitrogen supply. (See also Trial *A* on p. 167.)

*The water-insoluble phosphorous substances of the protoplasm*

The observations of p. 162 suggest that peptic digests of T.D.S. after drying as usual at 85–95° C. would he out of relation with what occurs in the animal digesting a natural graze. As a new plan, peptic digests at opt. conditions of the natural grass and of its undried "e. and c.w. and e. and b.w. insols.", showing digested protein-nitrogen, not in the usual way as a percentage of the total N in T.D.S., but as a percentage of the protoplasmic N, the latter determined by the aid of the Sap-volume Method as in the case of col. (4) of Table IV, have been tried. These studies are not completed. It is evident, however, that a considerable percentage of the protoplasmic proteins is very highly resistant[1] to the action of pepsin, probably greater the lower the ratio of "Complex *a*" to protein-containing complexes "rigid towards water" which include nucleoproteins, etc.

According to Jones (1914, p. 5), one of the large number of salts which polybasic nucleic acid and polyacid protein bases would be expected to form will have a greater resistance to pepsin than the others and will be formed as an end product of the action of pepsin upon any other of the salts having a greater proportion of protein. From this it would appear that the greater the removal of "Complex *a*" by natural peptic breakdown, the richer the residual protoplasm would become in nucleic acid and possibly also in other organic phosphorous substances such as phosphatides, etc. and water-insoluble earthy phosphates, causing as a net effect, an increasing ratio of phosphorus to nitrogen in the residues, i.e. higher in col. (5). It seems unlikely, however, that nucleoproteins would represent more than a very minor part of the protein-containing-

[1] Ames & Boltz (1912), "Effects of fertilisers on the composition of Alfalfa": "The quantities of pepsin-insoluble phosphorus which is combined with nitrogen as a highly insoluble compound and would be of doubtful value from a nutrition standpoint amounts to about 20% of the total phosphorus." (N.B. Calculated on the basis of protoplasmic phosphorus this figure would be much higher.)

complexes "rigid towards water" in such as the younger growths of Table IV. It is believed that in such cases the major part consists of Ca or Mg phosphate-protein-complexes and that some of these may have been separated in very prolonged "e. and c.w. treatments" (see below). Possibly when the protoplasm is exhausted to so great an extent as in the cases of cuts N and P the breakdown goes further than "Complex *a*" and extends to these to leave water-insoluble phosphatic products in the protoplasmic residues.

It seems evident that "Complex *a*" fluctuates as a protein reserve or store and that its content in the protein-containing-complexes of the protoplasm of grass fodders may be of high nutritional importance.

*Evidence of loosening, from the cells of P.Rg. leaves, of phosphorous substances and proteins together probably associated in some kind of water-insoluble "greater complex" on prolonging the "e. and c.w. treatment"*

Material used: cut Q of Table IV (2–4 in. leaf, cut 20 November).

The extent to which "e. and c.w." and "e. and b.w." treatments (p. 149) removed from the unpulverized fresh grass constituents other than those in the saps determined by the aid of the Sap-volume Method of p. 148 is shown below as percentages of the respective total quantities in the grass:

| | *Phosphorus* | Proteins (N × 6·25) | Non-nitrogenous organic matter | *Ash* |
|---|---|---|---|---|
| "e. and c.w. treatment" | 38·0 | 14·0 | 5·0 | −2·16 |
| "e. and b.w. treatment" | 12·0 | 0·9 | 2·4 | +4·42* |
| Extra by "e. and c.w." (diff.) | 26·0 | 13·1 | 2·6 | −6·58 |

* This figure for several soil-free P.Rg. leaf-growths was always similarly low. The actual estimates in this case for ash in "e. and c.w. insol.", "e. and b.w. insol." and saps on the above basis, as percentage of the total ash were 22·9, 16·32 and 79·26 respectively.

In this case the *first* day's procedure (p. 150) of the "e. and c.w." was continued for 4 days. On the 4th day both from 35 and 194 g. of the cut turbidity was first noticed in the torsion-liquors then quite neutral to delicate litmus paper. White deposits obtained from these liquors on standing responded positively to protein tests. It is regretted that they were not tested for phosphorus and that no attempt was made to exhaust the material of the white substance by continuing the treatment.

Chibnall *et al.* (1933) on treating grass with neat ether observed that in each cell the protoplasm had collapsed and shrunk to one end. It is suggested that after heat-coagulating the whole of the proteins in these

shrunken portions by the plunging into boiling water at the start of the "e. and b.w. treatment" only that part of the protoplasmic phosphorus in "Complex *a*" was soluble on completing the treatment. In the case of "e. and c.w." on the other hand, the shrunken portions whose proteins had not been heat coagulated, due to the effects of the torsioning, possibly assisted by slow enzymic change in one or other of the water-insoluble greater complexes, e.g. perhaps the splitting off[1] of phosphoric acid from the nucleic acid of nucleoproteins to some extent, eventually disintegrated after the removal of "Complex *a*" to yield water-insoluble complexes represented by the white deposit.

It is noted that the extra amounts of protein and non-nitrogenous organic matter removed by the "e. and c.w. treatment", viz. the 13·1 and the 2·6% respectively, are in somewhere about the same relation as in the general run of Chibnall-preparations from pasturages.

### *Distribution of protoplasm and phosphorus*

The evidence, (1) exudates represented the internal fluids as a whole (p. 149), (2) water-soluble protoplasmic phosphorus is sometimes quite low in young leaf growths when the sap phosphorus is high, while cell wall and vascular substances are not of such nature as to form loose chemical associations with water-soluhle phosphates nor to concentrate them on their surfaces, (3) so little of the total phosphorus of the grass was left in the "e. and c.w." loosening experiment even though undoubtedly the whole of the substance represented by the white deposit had not been removed, etc., all seems to point to the conclusion that the whole of the phosphorus of the grass which was not in the saps was in the protoplasm.

That this is probably true even also of stems, at any rate up to a moderately advanced development, seems likely from the fact that the dry solids of stems from a growth of P.Rg. at the stage of "inflorescences fully out of sheaths" contained very much less phosphorus nitrogen and ash than the dry solids of leaves from these same stems. Hence examinations of growing stems, etc. by these methods may show that phospborus not in the saps keeps close company everywhere with the proteins of the protoplasm.

[1] Probably the natural ferment splitting off phosphoric acid would remain with the nucleoproteins in spite of the most thorough extractions with cold water. The ferment was found by Jones (1914, p. 42) to follow thymus nucleoproteins through a purification process of alternate solution in dilute alkali and precipitation by acetic acid.

*Different behaviour of protoplasm on extracting the natural and oven-dried grass with water.*

The T.D.S. of six P.Rg. cuts of Table I, finely powdered after drying 48 hr. at 85–90° C., were extracted with (*a*) boiling water, (*b*) cold water with trituration. The whole of the phosphorons substances was soluble in all cases. On the other hand, the (sap-free) "e. and b.w. insols.", dried powdered and extracted with boiling water exactly as the T.D.S., rigidly retained their phosphorous substances (present to the degree shown in col. 6, Table IV) to a very large extent.

At present, the different behaviour of the T.D.S. is attribnted to changes brought about by the sap-acids, at the high concentration attained in the drying and under the prolonged influence of heat, in the phosphorous components (nucleic acid and probably phosphates of Ca or Mg, see p. 160) of the water-insolnble protein-containing-complexes of the protoplasm after heat coagulation, giving products soluble in the aqueous solution of acids, salts, etc. of the dry residues of the saps.

It is evident that examinations of extracts of such T.D.S. may mislead (see p. 149).

It is of interest that the T.D.S. of the untreated 4th growth of Table V, largely leaf but very sun-scorched and sered before cutting, dried at 85–90° C. and very finely powdered, on extracting with boiling water as above, gave a residue rigidly retaining phosphorous substances as if it were an ordinary "e. and b.w. insol." Probably phosphorous substances etc. in such sered material as graze would be locked to this extent against the animal's needs.

## Some effects of top-dressing perennial rye-grass with ammonium sulphate

*Trials A and B.* Unfortunately, other work prevented more than one cut after each dressing, "e. and b.w. treatments" were omitted from Trial A, and some of the outer oldest leaves died in Trial B. This dead leaf matter, impracticable to separate, confused the sap-volume determinations per unit weight of live grass. However, certain well-marked features of interest in the present problem are shown by the exudates which run out only from, and therefore represent, the live parts in such cases as Trial B.

*Trial A.* The 1st growth of Culture I in its 2nd year was cut on 21 May. One area was left untreated and another dressed in the evening of the same day with AS at 2 cwt. per acre. The cutting and dressing of the

latter area was repeated on 24 June and again on 9 August. A final cut on 11 September. Thus 2nd, 3rd and 4th growths of the season from the untreated and after one ($AS_1$), two ($AS_2$) and three ($AS_3$) successive dressings respectively.

*Trial B.* After improving the general fertility by giving Culture I on 8 April in its 4th year fine farmyard manure at about 9 tons per annum the 1st growth of its 5th year was cut on 2 June. Then one area untreated and two treated that day with AS at 3 and 6 cwt. per acre. Two months[1] later each was cut and on that day the treated were again treated respectively as before. After a further 2 months[1] each was again cut. Thus, 2nd and 3rd growths of the season from the untreated and after one "($AS_1$)" and two "($AS_2$)" successive dressings of 3 and 6 cwt. respectively.

Table V

| | Trial A | | | | | |
|---|---|---|---|---|---|---|
| | Untreated | | | Treated | | |
| Successive growths of the season | 2nd | 3rd | 4th | 2nd ($AS_1$) | 3rd ($AS_2$) | 4th ($AS_3$) |
| Date of cut | June 24 | Aug. 9 | Sept. 11 | June 24 | Aug. 9 | Sept. 11 |
| Approx. cwt. of T.D.S. per acre | 2·4 | 2·4 | 0·9 | 10·4 | 8·0 | 1·5 |
| % T.D.S. | 27·8 | 32·6 | 55·3 | 26·5 | 34·4 | 57·3 |
| In T.D.S. | | | | | | |
| % N | 1·74 | 2·28 | 2·51 | 1·93 | 2·6 | 2·98 |
| % "total $P_2O_5$" | 1·15 | 1·43 | 1·32 | 0·86 | 0·94 | 0·75 |
| ml. of saps per g. of T.D.S. | 2·75 | 2·21 | * | 2·95 | 2·21 | * |
| In 100 ml. of saps: | | | | | | |
| Dry solids, g. | 9·7 | 10·72 | — | 10·01 | 13·44 | — |
| mg. N | 67 | 113 | — | 68 | 158 | — |
| mg. "total $P_2O_5$" | 246 | 320 | — | 143 | 186 | — |
| Titration,† ml. of $N/10$ alk. to phenolphthalein | | | | | | |
| +25 vols. $H_2O$ | 54·3 | — | — | 32·6 | — | — |
| +50 vols. $H_2O$ | — | 65·1 | — | — | 36·9 | — |
| In saps per g. of T.D.S. | | | | | | |
| mg. N | 1·85 | 2·5 | — | 2·0 | 3·3 | — |
| mg. "total $P_2O_5$" | 6·8 | 7·1 | — | 4·2 | 3·9 | — |
| Sap phosphorus as % of total phosphorus | 59·13 | 48·24 | — | 49·65 | 41·49 | — |

* No exudates (saps) obtainable. Almost completely sered by sun-scorching.
† See p. 164.

The $p$H and titratable acidity were determined after the fresh exudates stood 24–27 hr. at R.T.; ether present; stoppered. Hence

[1] Through drought there was practically no growth in the earlier parts of these periods. Actual growth periods were about 5 and 6 weeks respectively during which the conditions were moderately moist. A total of 1·1 in. of rain, with a little almost on every day, fell during the 14 days preceding the cuttings of 5 October.

$[H^+]$ and acidity values were perhaps a little on the high side.[1] However, the standing period was practically constant.

Table VI

| | Untreated | Trial B: Untreated | | Trial B: The 3 cwt. | | Trial B: The 6 cwt. | |
|---|---|---|---|---|---|---|---|
| Successive growths of the season | 1st | 2nd | 3rd | 2nd ($AS_1$) | 3rd ($AS_2$) | 2nd ($AS_1$) | 3rd ($AS_2$) |
| Date of cut | June 2 | Aug. 5 | Oct. 5 | Aug. 5 | Oct. 5 | Aug. 5 | Oct. 5 |
| Approx. cwt. of T.D.S. per acre | 18 | 3·3 | 1·7 | 17·2 | 11·1 | 28·6 | 12·7 |
| % T.D.S. | 23·3 | 35·2* | 29·8* | 30·1* | 24·8 | 28·5* | 23·5 |
| In T.D.S. | | | | | | | |
| % N | 1·27 | 1·85 | 2·6 | 2·09 | 3·35 | 2·40 | 3·92 |
| % "total $P_2O_5$" | 0·84 | 1·04 | 1·20 | 0·99 | 1·26 | 1·06 | 1·20 |
| ml. of saps per g. of T.D.S. | 3·40 | (1·92) | (2·48) | (2·40) | 3·16 | (2·59) | 3·35 |
| In 100 ml. of saps: | | | | | | | |
| Dry solids, g. | 7·27 | 8·7 | 9·13 | 6·92 | 7·43 | 6·07 | 6·79 |
| mg. N | 49 | 82 | 81 | 84 | 119 | 142 | 154 |
| mg. "total $P_2O_5$" | 147 | 271 | 236 | 212 | 195 | 210 | 159 |
| Titration, ml. $N/10$ alkali to phenolphthalein | | | | | | | |
| Undiluted | 54 | — | 70 | — | 49 | 50 | — |
| +10 vols. $H_2O$ | 43 | 62 | 61 | 40 | 43 | 35 | 38 |
| +50 vols $H_2O$ | 38 | 51 | 51 | 35 | 39 | 31 | 37 |
| *p*H of saps: | | | | | | | |
| Undiluted | 4·8 | 4·8 | 5·0 | 5·3 | 5·3 | 5·5 | 5·3 |
| +10 vols. $H_2O$ | 5·7 | — | 5·5 | — | 5·8 | — | 5·8 |
| +50 vols. $H_2O$ | 6·2 | — | 5·8 | — | 6·1 | — | 6·1 |
| In saps per g. of T.D.S. | | | | | | | |
| mg. N | 1·7 | (1·6) | (2·0) | (2·0) | 3·7 | (3·7) | 5·2 |
| mg. "total $P_2O_5$" | 5·0 | (5·2) | (5·8) | (5·0) | 6·2 | (5·4) | 5·3 |

* Contained a good deal of dead leaf (see p. 162). Dead leaf solids in T.D.S. of the Untreated of 5 October were somewhere about 20%. The two treated of 5 October however had very little dead. Figures in brackets would all be higher if they could be calculated on a dead-free basis.

*p*H: Colorimetric. Preferred to electrometric because of the adverse effects in the latter of possible traces of nitrates.

*Titration to phenolphthalein.* 1 or 2 c.c. portions in 250 Erlen. over a white plate. The undiluted: not sharp. The diluted: exceedingly sharp. (Alkali even as weak as $N/50$ gives a very abrupt end-point colour change in suitably diluted exudates.)

[1] Exudates kept aseptic by ether in tightly stoppered vessels progressively increase in acidity to phenolphthalein up to maxima probably somewhat quicker at 37° C. than at R.T., and to a much greater extent for W.W.C. than P.Rg. The net formol value (i.e. minus the acidity value) also increased for W.W.C., e.g. respective increase in the acidity and net formol at 37° C. for (*a*) a young leaf growth of W.W.C.: to maxima, by 88 and 19% in 4–5 days, (*b*) P.Rg., cut Q of Table III: 30 and 0% in 2 days.

*Notes to Tables V and VI*

(1) *Trial A*. Severely droughted (cf. the columns referred to on p. 150). *Trial B*. Conditions moderately moist (see footnote to p. 163).

(2) Remarkable progressive falls[1] in [H+] and titratable acidity per unit volume accompanied *increasing dilution* of exudates (or "saps") by water. (N.B. Dilutions must be equal for such values to be comparable.)

(3) The lower the titratable acidity, the lower the [H+] and the lower the phosphate content, in the saps.

(4) Titratable acidity [H+] and phosphates in the saps and in saps per g. of T.D.S. are higher the lower the yield of produce.

(5) A.S. very greatly increased the yield of produce and hence of protoplasmic proteins; in spite of lack of water through drought in Trial A.

(6) More nitrogen but remarkably less phosphates in the saps and in saps per g. of T.D.S., and very much greater yields of protoplasmic proteins per unit area in the A.S. treated growths of Trial A.

Similar remarks apply to Trial B save that lowness of phosphates in the saps is not much in evidence. Apparently moister conditions and extra from the residues of the *farmyard manure applied in the previous season (p. 163) had favoured phosphate supply by* the soil.

(7) The two dead-free A.S. treated 3rd growths of Trial B (see footnote to Table VI), gave very high percentages of protoplasmic nitrogen and phosphorus "sol. in the e. and b.w. tr. not in the saps" and low phosphorus per g. of protein in the "e. and b.w. insol." (see data at the foot of Table IV.)

*Discussion arising from Tables V and VI*

As the general trend, progressively with successive and, within limits, *bigger successive dressings, AS gave much higher yields of produce* richer in protoplasmic proteins, with higher nitrogen and lower titratable acidity [H+] and phosphates in the saps.

Undoubtedly the *p*H of P.Rg. saps may vary to some extent in varying circumstances of growth. In Trial *B* and, judging by low titratable acidity, also in Trial *A*, the AS treated were 0·3 to 0·7 higher. Within this zone, low [H+] in saps seems to favour protein building in protoplasm.

*Note* (3). Precipitates by 9 vols. of 97% alcohol to P.Rg. exudates, washed with 90% alcohol, dried in desiccator, and extracted with water gave 5–10% of their weight as water-insoluble residues containing 20–26% $P_2O_5$. The phosphorus distribution after this treatment in the case

[1] Exudates of various pasturages, including W.W.C., all behaved like this. Apparently, washing out vacuole matter by water in the protein preparation processes (p. 136) would *thus the more rapidly alter the environment* of the protoplasm to one of low [H+]. On the other hand, since the *p*H of saturated, 6 and 1% solutions of ammonium sulphate (A.R.), colorimetrically, came out at about 5·0, 5·2 and 5·4, and P.Rg. exudates generally at about 5·2, it appears that washing out vacuole matter by saturated ammonium sulphate as in the process of p. 142 was done without reducing the [H+], perhaps thus avoiding wastage of the "easily resolved greater complex" (see pp. 156 and 170).

of the 2nd and 3rd growths of Trial *A*, as percentages of the total exudate phosphorus, is shown below:

| | | Trial A | | | |
|---|---|---|---|---|---|
| | | Untreated | | Treated | |
| Growths of Table V: | | 2nd | 3rd | 2nd ($AS_1$) | 3rd ($AS_2$) |
| *Alcohol precipitate* | | | | | |
| water-insoluble residue | (1) | 39·7 | 36·2 | 51·0 | 47·8 |
| aqueous extract | (2) | 54·6 | 59·2 | 43·7 | 46·4 |
| Alcohol filtrate | (3) | 5·7 | 4·6 | 5·3 | 5·8 |

The acidity of these aqueous extracts, to phenolphthalein, varied with their phosphate content and also, although relatively much smaller, with the acidity and phosphate content of the whole exudates. Evidently this considerable part [line (2)] of the sap-phosphates had been precipitated by alcohol as primary phosphates of K or Na. The higher the concentration of phosphates in saps and the nearer the *p*H to about 4, the higher the concentration of primary phosphates and acidity to phenolphthalein, thus affording some explanation of Note (3). The acidity to phenolphthalein of the Trial A exudates however was about twice as great as primary phosphates corresponding to their phosphate content and *p*H could account for. These studies continue.

Line (2) shows a considerably lower proportion of the primary phosphates of K or Na for the saps of the AS treated (see p. 167).

Apparently differing amounts of nitrogenous bases such as ammonia, certain amino acids, etc., could greatly modify the situation. Preliminary examinations by the "Alcohol Methods" (Foreman, 1928) indicate increased volatile bases and amino acids for the saps of AS treated of Trial A. Judging from sap-nitrogen per g. of T.D.S. in Table VI greater increases will be shown on examining available solutions prepared from the saps of Trial B. Possibly, better "nitrogen" supply by the soil and greater protoplasmic protein production entail the presence of the nitrogenous bases in the saps in sufficient amounts for keeping [$H^+$] favourably low.

### *Quantitative relations between sap-phosphates and protoplasmic protein building*

Calculations show that the greater bulk of produce from AS treated than untreated in Trial A had 3·2–2·2 times the total phosphorus and 2·2–2 times the total sap-phosphates per unit area. Probably this and also more water for developing the bigger growth in spite of the drought

were due to deeper root range for bigger plants. Although there was this extra phosphate per unit area from the soil, yet sap-phosphate values per g. of T.D.S. were only 60–55% of *those for the corresponding* untreated. Hence, demands of developing protoplasm for phosphates from the saps, prior to stoppage of growth by high concentration of saps caused by the drought (see p. 156), had outpaced the capacity of the soil, impoverished previously by several cuts and in the adverse circumstance of too little water, to supply phosphates to the saps. *It is seen, however, that* although sap-phosphates per g. of T.D.S. were only the 60–55%, yet the concentration of sap-phosphates ($P_2O_5$ per 100 ml.) is much the same as for the active young growths A to H of Table III. Probably this was partly due to further loss of water after the stoppage of growth but it also seems likely that prior to the stoppage loss of water had kept the sap-phosphate concentration above a possible no-growth-level.

On the other hand, even with the soil supplying less phosphate per unit area to the untreated, the sap-phosphate concentration and sap-phosphates per g. of T.D.S. were both quite high and almost twice as great *as in the AS-treated. Hence in this case it seems clear that* very poor "nitrogen" supply by the soil had been the chief limiting factor, but the very poor supply of phosphates from soil to saps was much more than sufficient for meeting the demands of the very small development of protoplasm.

*Owing to the omission of "e. and b.w. treatments" it was not* possible to examine the protoplasm of these severely droughted growths of Trial A, like M and O in Table IV, for breakdown of the protein-phosphate-complex. It is evident, however, from the extremely low sap-phosphates per g. T.D.S. in the AS-treated that the breakdown products had not been *discharged into saps and that the make-up of the sap solids* was practically as left when protein-building was stopped by high general concentration of saps (see p. 156). The lower proportions of phosphates of K or Na in the saps mentioned on p. 166, suggests that a greater proportion of these had been withdrawn by the protoplasm to render it richer in the "*easily resolved greater complex*". *Note* (7) *indicating* extensive breakdown of this "greater complex" in the two AS-treated of Trial B supports the view that AS dressings are conducive to richness of the protoplasm in this respect. Since in this case the conditions were moderately moist (Note (1)) and the saps moderately dilute (see dry solids per 100 ml. in Table VI) perhaps some *of the breakdown products* had been received by the saps and the breakdown changes were even more extensive than col. (4) of Table IV indicates. Since however the dressings

were of AS only and very excessive the possibility of abnormal behaviour on the part of these two growths must be entertained.

*Conclusion.* These observations afford further illustrations of quantitative relations between "nitrogen" and phosphates in the saps and production of protein-phosphate-complexes in the protoplasm. AS dressings to the growth giving saps richer in "nitrogen" and possibly sap-phosphates richer in those of K, Na or "$NH_4$" caused larger utilization of such sap-phosphates as were available in greater protein-phosphate-complex production richer in "Complex *a*".

### Selected general provisional conclusions

1. Proteins occur in the protoplasm of the permanent cells of the P.*Rg.* leaf as "greater complexes" in which they are chemically associated with water-soluble and water-insoluble phosphorous substances, probably phosphates chiefly.

2. Building and breakdown of "greater complex" as well as protein component alternate frequently during growth in relation to the weather and its effects.

3. After the building and breakdown the protoplasm is richer and poorer in that greater complex, viz. "Complex *a*", whose phosphorous component, probably chiefly the phosphates of K or Na, is water-soluble.

4. At the stage when leaf increase stops and existing protein resources are drawn upon for developing inflorescence and seed, the breakdown, to the greater extent the more starved the grass for "nitrogen" by the soil, may extend to one of the greater complexes "rigid towards water", probably Ca or Mg phosphate-protein, believed to occur with "Complex *a*" in the cytoplasm.

Protoplasm has often been regarded as containing a semi-rigid framework and a less viscous part. Quoting Seifriz (1936, p. 266):

"Whether we turn to newer work on the physiology of the cell or to older work on cytology we find support for a semi-rigid framework in protoplasm."

"The older workers in cytology held similar opinions expressed in the 'spongioplasm' (framework) and 'hyaloplasm' (intervening fluid) of Leydig and the 'ground substance' and 'reticulum' of Carnoy and others. E. B. Wilson states that the 'continuous substance' (i.e. spongioplasm) is the most constant and active element and that which forms the fundamental basis of the protoplasmic system to which E. G. Conklin

agrees in saying that the protoplasm is composed of a more fluid and a more viscous portion."

It seems possible for the less and more viscous parts of the cytoplasm to consist largely of K or Na phosphate-protein, viz. "Complex *a*" and Ca or Mg phosphate-protein respectively. "Ca coagulates or aggregates the surface protoplasm while Na has no influence" and "Ca raises the viscosity of protoplasm while Na lowers it", etc. (Seifriz, 1936, p. 221).

5. *Protein reserve in leaf protoplasm.* Protein in the protoplasm in the form of "Complex *a*" fluctuates during growth as a "reserve" or "store" broken down at one time to meet the need of the breakdown products at the growing points and replenished in other circumstances at another.

6. The saps lose and gain nitrogenous materials and phosphates, etc. in relation to the requirements for the formation of the "greater complexes" and excretions after their breakdown respectively.

7. "*Balance*". The evidence in general, especially mentioning in this connexion growths limited by poor "nitrogen" supplies from the soil, viz. cuts N and P and the untreated of Trial *A* (see discussions on pp. 158 and 167) seems clearly to suggest that in order to attain the highest rate of net production of "greater complexes" and, provided the building is the last occurrence before cutting, the highest content of "Complex *a*", nitrogen, phosphates, KNa, CaMg, etc. must be supplied by the soil in such forms and quantities as shall be in relation with the saps providing the protoplasm with nitrogenous materials, phosphates, etc. in favourable proportions for meeting the construction of the greater complexes. Thus the results of these studies suggest a fundamental basis for the term "balance" often used in discussing problems of manuring and the methods seem suitable for elucidating other aspects of supply and demand in growth processes.

8. The relative quantities of essential mineral radicles supplied by a soil probably vary with varying weather.

9. The content of "Complex *a*" in the protein-containing-complexes of the protoplasm varied with protein production and breakdown during growth, the stage of growth in relation to the "purposes" of the grass, varying weather in relation to the conditions determining the production and breakdown and the capacity of the soil to provide essentials in suitable forms and relative amounts.

10. The content of "Complex *a*" determines the yield of "soluble proteins" by the Chibnall-methods.

11. *Dispersal of protoplasmic protein into aqueous solution.* Two types

of "greater complex" in the protoplasm of P.Rg. may be distinguished according to their behaviour on changing the environment from vacuole fluid to plain water, viz. "Complex *a*", in which the protein is loosely combined with phosphates of KNa only at high enough [$H^+$] and in the presence of similar phosphates at high enough concentration, both of which conditions the vacuole fluids fulfil, and "Complexes *b*", associations "rigid towards water", including nucleoproteins and probably Ca or Mg phosphate-protein, which remain intact and insoluble even if freed from the sap-free cells into plain water in the pulping process of the Chibnall-methods, and in spite of the large reductions in [$H^+$] and dissolved phosphates the change from vacuole fluid to plain water entails. Apparently both types may be further complicated to some extent by organic non-nitrogenous organic matter possibly as another component.

It is suggested that whatever quantity of "Complex *a*" is amongst the protoplasmic matter the cells part with on pulping the sap-free material with water, varying with the differing effects of ether, ether-water or "used" ether-water (p. 136), now in the absence of sap-phosphates and at the much lower [$H^+$] of *p*H 7, is completely dissociated into its components which both disperse into solution, and, on the addition of HCl up to the isoelectric point, the precipitate consists or very largely consists of the protein component and not "Complex *a*" because of the nature of the particular phosphate (*vide* p. 168) and its extremely low concentration in the bulky solutions.

On this basis varying yields of preparations of the soluble proteins even after employing "used" ether-water may correspond with varying quantitative ratios of "Complex *a*" to "Complexes *b*". It is believed that the values in col. (8) or (9) of Table IV, provided there has been no retention of breakdown products in the protoplasm (p. 156), reflect the full quantities of "Complex *a*" and perhaps the limits of the "Soluble Protein" preparations.

12. *Circumstances of growth and varying content of "Complex* a" *in the protein-containing-complexes.* Apparently the ratio of "Complex *a*" to "Complexes *b*" in the protoplasm could vary with varying ratio in the vacuole fluids of KNa to CaMg either as phosphates or in such forms as will surrender these bases to the phosphate-protein-complexes formation. Presumably the relative quantities of different salts of each of these bases, e.g. sulphates, chlorides, phosphates, salts of organic acid radicles, in the saps must be influenced by the inorganic forms in which these bases were taken up from the soil and, in turn, by the kind of soil, the weather and manuring in so far as these can determine what is dissolved in the soil water from time to time.

Accurate analytical data by methods of such nature, e.g. those which avoid heating and its effects, etc., as to determine the true natural make-up of vacuole solutions and a clear view of the actual acids and bases contributing in the equilibria from time to time during growth are greatly needed. Fortunately exudates by Chibnall's original ether method, representing the internal fluids as a whole (p. 149), afford the opportunity.

In regard to different species of plants it appears that inherent differences in the make-up of the saps might correspond with considerable differences in the situation as set out above. Hutchison and Mottram (1933) quote data "from a table by Esbach" showing that spinach ranks with rhubarb and sorrel in containing very large amounts of oxalic acid (parts per 1000: spinach, 1·91–3·27; rhubarb, 2·466; sorrel, 2·74–3·63; four other species between 0·002 and 0·052). Conceivably the oxalic radicle would tend to curtail the supply of Ca and Mg available for the building of Ca or Mg phosphate-protein-complex either by their previous separation or unsuitable presentation as oxalates, and thus account for the relatively very high yields of the "soluble proteins" obtainable from spinach (see p. 135). It seems likely that much further enlightenment might follow studies of spinach on the lines herein described.

## GENERAL SUMMARY

1. Studies lead to the conclusion that Chibnall-preparations of pasturage proteins, in spite of their small amounts of carbohydrate impurity, may yet yield, on hydrolysis by mineral acids, practically the right amounts even of most of those amino-acids which are adversely affected by carbohydrates to some extent when tested singly under the conditions.

2. Preparations made in another way after treating the pasturage with ether as in the original Chibnall-method showed features of interest in the problem of attaining very pure proteins now much needed for purposes of comparison.

3. A new method is introduced by which it is possible to compare what may be extracted from the protoplasm of fresh unpulverized pasturages with the composition of the natural saps on a unit weight basis.

4. Production and breakdown of protoplasmic protein in perennial rye-grass during growth consistently appeared related to variations in the content of "nitrogen" and phosphates in the saps and the supplies of "nitrogen" and phosphates from the soil.

5. Proteins occur in the protoplasm of perennial rye-grass as "greater complexes" in which they are associated with phosphorous substances.

6. Two types of "greater complexes" are distinguished by their behaviour towards water, viz. "Complex *a*", containing water-soluble phosphorous substances, probably phosphates of K or Na chiefly, and "Complexes *b*", "rigid towards water", probably including Ca or Mg phosphate-protein as part of the cytoplasm.

7. The saps lose and gain nitrogenous materials and phosphates in relation to requirements in the production and excretions after the breakdown of the "greater complexes" of the protoplasm.

8. The content of "Complex *a*" in the protein-containing-complexes of the protoplasm varies with (1) protein production and breakdown during growth, (2) stage of growth in relation to the sequence of events in the life history of the grass, (3) conditions in the grass as determined by the weather, (4) the relative amounts of mineral radicles supplied by the soil in varying weather and manuring.

9. "Complex *a*" fluctuates as a protein reserve or store and its content in the protein-containing-complexes of the protoplasm of grass fodders may be a matter of considerable nutritional importance.

10. The results suggest a fundamental basis for the term "balance" often used in discussing problems of manuring and the methods seem suitable for elucidating other aspects of supply and demand in growth processes.

11. Probably the content of "Complex *a*" in the protein-containing-complexes of the protoplasm of grasses determines the yield of the "soluble proteins" from the Chibnall-methods.

The opportunities afforded by the work of Chibnall and colleagues are appreciated.

Finally, I have pleasure in acknowledging the able and careful help of Mr B. J. Constable in carrying out these experiments.

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# READILY SOLUBLE MANGANESE OF SOILS AND MARSH SPOT OF PEAS

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THE defect in pea seeds commonly known as Marsh Spot has lately been the subject of a number of investigations. The earlier results showed the disease to be non-parasitic (Lacey, 1934) and not related to potassium deficiency or manurial treatment in general (De Bruin, 1933; Ovinge, 1935). The more recent papers dealing with the cause of the defect have focused attention on the soil conditions. In a survey of the occurrence of Marsh Spot in Holland, Ovinge (1935) showed that the trouble was generally found on alkaline soils and on relatively young polders. In a similar but more detailed survey of the Romney Marsh area in England, Furneaux & Glasscock (1936) were able to establish a correlation between the intensity of Marsh Spot damage and soil type, taken in conjunction with the height of the water table. Quite recently it has been suggested that the disease may be due to a deficiency of available manganese (Löhnis, 1936; Pethybridge, 1936).

Through the kindness of Mr Furneaux the writer obtained representative soil samples from his survey for laboratory investigations on the more readily soluble manganese.

## THE MARSH SPOT SOILS

Furneaux & Glasscock (1936) described five soil series in Romney Marsh which are represented in the present investigation by samples from thirty-five fields. From the summary in Table I of the main features of the soil series it will be seen that the Marsh Spot damage is most severe on heavy soils with seriously impeded drainage.

Table I. *Soil series in order of increasing severity of Marsh Spot*

| Soil series | Surface texture | Deep subsoil texture | Drainage |
|---|---|---|---|
| New Romney (NR) | Fine sandy loam | Sandy loam | Good |
| Finn (F) | Loam | Sandy loam | Slightly impeded |
| Brenzett (B) | Silt loam | Silt loam | Slightly impeded |
| Ivychurch (I) | Silty clay loam | Fine sandy loam | Impeded |
| Newchurch (N) | Silty clay loam | Clay loam | Poor |

It is to be expected that the progressive change in soil texture and drainage through the soil series in the above table would be reflected in the soil reaction, and in the amounts and forms of the soil manganese. Indeed, for the soils investigated from Romney Marsh the occurrence of Marsh Spot is more closely related to soil reaction than to soil texture. By grouping the soils according to soil texture and examining the distribution of Marsh Spot, as in Table II, it is seen that although Marsh Spot occurred on three-quarters of the heavy and medium soils it also occurred on a quarter of the light soils. When a similar grouping is made for soil reaction by dividing the soils at *p*H 7·0 it is found that Marsh Spot occurred on four-fifths of the alkaline soils but never on an acid soil.

Table II. *Marsh Spot on Romney Marsh soils in relation to soil texture and reaction*

| | Soil texture | | Soil reaction | |
|---|---|---|---|---|
| | Medium and heavy | Light | *p*H 7·0 or over | *p*H below 7·0 |
| Soil Series ... | N, I, F, B | NR | — | — |
| Marsh Spot present | 15 | 4 | 22 | 0 |
| Marsh Spot absent | 7 | 9 | 5 | 8 |

By dividing the soils both by soil reaction and soil series, as in Table III, it will be seen that the differences between soil series are intimately associated with differences in soil reaction. Half of the light soils in the New Romney series were acid, and were free from the defect, whereas only two of the twenty-two heavier soils were acid. Only four of the twenty alkaline medium and heavier soils were free from the disease. Among the alkaline soils there was little evidence that the soil series was of importance. Table III also contains the distribution of Marsh Spot in relation to previous cropping. This table and a more extensive one, including other centres not represented here by soil samples, agree with Furneaux & Glasscock's finding that there was no clear relationship between previous cropping or manuring and the severity of the effect. On alkaline soils the disease was somewhat less frequent after cereals or peas than after other crops.

The fact that the disease does not occur on acid soils adds to the likelihood of a manganese deficiency, for Grey Speck disease of oats and other instances of manganese deficiency are known to be restricted to neutral or alkaline soils.

Table III. *Marsh Spot in relation to soil texture, soil reaction and previous cropping*

| | | Alkaline soils pH 7·0 or over Marsh Spot | | Acid soils pH below 7·0 Marsh Spot | |
|---|---|---|---|---|---|
| | | Present | Absent | Present | Absent |
| (1) Romney Marsh soils: | | | | | |
| Soil texture | Soil series | | | | |
| Heavy | I and N | 9 | 4 | 0 | 1 |
| Medium | B and F | 7 | 0 | 0 | 1 |
| Light | NR | 5 | 2 | 0 | 6 |
| (2) Romney Marsh soils: | | | | | |
| Previous crops | | | | | |
| Fallow or roots | | 9 | 3 | 0 | 0 |
| Cereals or peas | | 9 | 1 | 0 | 2 |
| Pasture or leys | | 3 | 1 | 0 | 6 |
| (3) Other Kent soils | | 1 | 10 | 0 | 7 |

## DETERMINATION OF READILY SOLUBLE SOIL MANGANESE

It is to be expected that the solubility of the manganese in soils will depend both on soil reaction and on the oxidation-reduction conditions. In acid soils some of the manganese will be held normally as an exchangeable base, but salt solutions may also extract manganese which is loosely held by organic matter or which is reduced from the oxides during extraction. In the majority of alkaline soils the more mobile manganese will occur mainly as some form of manganese dioxide, but unpublished work suggests that in some alkaline soils, rich in organic matter, manganese may be held in an insoluble form by combination with the organic matter. The distinction between these forms has proved to be a matter of great difficulty, and the determination of the exchangeable form is much more difficult than for the more common cations.

Piper (1931) was of opinion that the available form was the bivalent manganese but not the higher oxides. Leeper (1935) concluded that the amount of a certain kind of manganese dioxide, characterized by its capacity for oxidizing hydroquinone at a certain *p*H, was of importance in determining whether a soil was manganese-deficient or not.

A valuable contribution to the problem of the availability of soil manganese was made by Steenbjerg (1933, 1934), who worked with a great variety of arable Danish soils on which Grey Speck disease of oats sometimes occurred as a result of overliming. By determining the rate of extraction by a salt solution and extrapolating to estimate the total amount of extractable manganese, he was able to predict with some confidence which soils might be liable to cause Grey Speck trouble after liming. In Steenbjerg's method for determining readily soluble man-

ganese the soil was leached on a Büchner funnel with normal magnesium nitrate solution, the leachates being collected successively in measuring flasks and analysed for manganese by Marshall's persulphate method (1901). The amount of manganese ($y$ in mg. Mn per 100 g. soil) extracted by leachates ($x$ in in units of 100 c.c.) at successive stages in the extraction were combined in the hyperbolic expression,

$$y=\frac{xS}{x+qS} \quad \text{or} \quad \frac{1}{y}=\frac{1}{S}+\frac{q}{x},$$

to give an estimate of the total amount of extractable manganese ($S$ in mg. Mn per 100 g. of soil) and the firmness with which the manganese was held ($q$). High $q$ values denote slow extraction of the manganese and are taken to imply low availability.

The application of the Steenbjerg method may be illustrated in Table IV for a number of soils known to be manganese-deficient. Soils 1, 2 and 3 were from untreated plots in an experiment of W. Morley Davies in Warwickshire, in which oats suffered from Grey Speck, and soil 4 was from a plot where the disease had been controlled. Sample 5 was from another Warwickshire field, known to give rise to Grey Speck in oats, and where sugar beet showed, at the time of sampling, a characteristic yellowing of the leaves ("Speckled Yellows"). Sample 6 was from a plot in the same field on which the yellowing had been cured, at least temporarily, by the addition of manganese sulphate. Samples 7 and 8 were from Dutch fields on which oats suffered from Grey Speck disease. Each of the soils known to be manganese-deficient had either no readily soluble manganese or a very small amount which was firmly held (i.e. low $S$ and high $q$ values). The two soils (samples 3 and 6) to which manganese sulphate had been added several months previously contained appreciable amounts of readily soluble manganese.

Table IV. *Readily soluble manganese (mg. Mn per 100 g. soil) by Steenbjerg's method*

| | Untreated plots | | | | Plots treated with Mn $SO_4$ | | | |
|---|---|---|---|---|---|---|---|---|
| | Soil | *p*H | *S* | *q* | Soil | *p*H | *S* | *q* |
| Warwickshire: | | | | | | | | |
| Oats | 1 | 7·1 | 0·00 | — | 4 | 6·9 | 0·72 | 7·8 |
| Oats | 2 | 7·2 | 0·12 | 21 | — | — | — | — |
| Oats | 3 | 7·6 | 0·00 | — | — | — | — | — |
| Sugar beet | 5 | 7·6 | 0·00 | — | 6 | 7·0 | 1·02 | 0·9 |
| Holland: | | | | | | | | |
| Oats | 7 | 6·9 | 0·12 | 18 | — | — | — | — |
| Oats | 8 | 7·6 | 0·35 | 81 | — | — | — | — |

Similar results to the above were obtained for a pair of Lincolnshire silt soils. In the lower part of a field some 35 % of pea seeds were affected by Marsh Spot, whilst none of those in the upper part was affected. The analytical results which are given in Table V showed that there was no readily soluble manganese in the lower part of the field, whilst there were small amounts of readily soluble manganese in samples from each of several depths in the upper part of the field, which was free from Marsh Spot.

Table V. *Readily soluble manganese (mg. Mn per* 100 *g. soil) in Lincolnshire silt soils*

| | Without Marsh Spot | | | | With Marsh Spot | | | |
|---|---|---|---|---|---|---|---|---|
| | pH | *S* | *q* | $MnO_2$ mg. per 100 g. soil | pH | *S* | *q* | $MnO_2$ mg. per 100 g. soil |
| 0–6 in. | 7·6 | 0·3 | 60 | 0 | 8·0 | 0 | — | 0 |
| 6–16 in. | 7·2 | 0·2 | 50 | 0 | 7·6 | 0 | — | 0 |
| 2 ft. | 7·0 | 0·2 | 60 | 0 | 7·2 | 0 | — | 0 |

Analyses by the Steenbjerg method for the readily soluble manganese of Kent soils, which will be discussed more fully later, showed that the acid soils, on which peas were free from Marsh Spot, generally contained more readily soluble manganese than the alkaline soils on which pea seeds were affected, but there were a number of exceptions.

## Other methods for determining the readily soluble manganese

The Steenbjerg method of leaching soils on a Büchner funnel and considering the rate of extraction was developed for Danish soils of a more uniform texture than those encountered in this work. The method is open to the objection that the rate of extraction involves the permeability of artificially packed soil and the speed of diffusion through crumbs of soil. Extractions were therefore made under more uniform and controlled conditions by shaking 10 g. of soil mechanically with 250 c.c. of 1 $N$ (or 0·05 $N$) calcium nitrate solution and determining the manganese in the extract. Two different concentrations were employed in order to obtain some expression for the ease of displacement of the soil manganese. Manganese was also determined by treating 10 g. of soil with successive small lots of $M/2$ acetic acid, stirring and decanting to give 500 c.c. extract, as in the Rice Williams's (1931) method for exchangeable bases. In addition, 10 g. of soil were shaken mechanically for 24 hr. with 100 c.c. of 1 % citric acid.

Most of the Kent soils were manganiferous in the sense that they contained free manganese dioxide. Approximate estimations of the amounts of manganese dioxide were made by Feigl's (1921) colorimetric benzidine method. *p*H determinations were made either by the glass electrode or colorimetrically, the quinhydrone electrode being unsuitable for such soils (Heintze, 1934).

## Manganese and Marsh Spot

Since only small samples were available from many of the fields, it was not possible to analyse every soil by each of the above methods, and in the statement of results in the Appendix the soils are grouped for convenience according to the analytical methods used. The Appendix contains all the analyses of readily soluble manganese determined by salt extractions, together with the soil reactions and approximate estimates of the manganese dioxide contents. It further gives the severity of the Marsh Spot damage, expressed as the percentage of peas affected in the field, and, in addition, an estimate of the susceptibility of the pea variety concerned based on Furneaux & Glasscock's trials at Wye. The Romney Marsh soils are grouped according to soil reaction, the acid ones being free from the disease and the alkaline ones being further subdivided into groups with and without Marsh Spot respectively. Since the soil samples available from outside Romney Marsh included only one centre known to be affected by the disease, they have been separated from the group of Romney Marsh soils without Marsh Spot. The data in the Appendix may be considered in a condensed form as the $2 \times 2$ tables in Table VI, which shows the occurrence or absence of Marsh Spot in soils grouped according to their readily soluble manganese contents as determined by salt extractions. A higher limit of readily soluble manganese was taken for the analyses made by extracting with $1N$ calcium nitrate solution, since most soils yielded more manganese by this extraction than by leaching with $1N$ magnesium nitrate solution.

The data in Table VI show clearly that there is an obviously significant correlation between the occurrence of disease and the readily soluble manganese over all soil samples from Kent. Thus, for thirty-seven soils analysed by the calcium nitrate method there was no instance of diseased peas on soils with more than 3 mg. Mn %, whilst peas free from Marsh Spot occurred on only three soils with less than this amount of manganese. When the comparisons are restricted to soils within the Romney Marsh area, several soils rich in readily soluble manganese and free from the disease are eliminated, but the correlation is still significant. The fact

that none of the acid soils, but most of the alkaline ones gave peas affected by Marsh Spot prevented valid comparisons within the acid or alkaline groups of Romney Marsh soil treated separately. It is therefore only by virtue of the sharp distinction between acid and alkaline soils that the results from the Romney Marsh soils investigated support the conclusion that Marsh Spot is due to manganese deficiency. Other factors, as, for example, calcium supply, associated with soil reaction conditions, rarely result in so sharp a break near the neutral point, and the data may therefore be regarded as in harmony with the view that the reaction of Romney Marsh soils affects the pea crop mainly through the solubility of the soil manganese. There are, however, a few notable exceptions.

Table VI. *Distribution of Marsh Spot (M.S.) in relation to readily soluble manganese in Kent soils*

| | Steenbjerg's magnesium-nitrate extracts *S* mg. Mn % | | 1 *N* calcium-nitrate extracts mg. Mn % | |
|---|---|---|---|---|
| | 0–2 | Over 2 | 0–3 | Over 3 |
| All Kent soils: | | | | |
| M.S. absent | 3 | 10 | 3 | 15 |
| M.S. present | 15 | 1 | 19 | 0 |
| All Romney Marsh soils: | | | | |
| M.S. absent | 3 | 6 | 3 | 8 |
| M.S. present | 14 | 1 | 18 | 0 |
| Alkaline Romney Marsh soils: | | | | |
| M.S. absent | 2 | 2 | 2 | 2 |
| M.S. present | 14 | 1 | 18 | 0 |
| Acid Romney Marsh soils: | | | | |
| M.S. absent | 1 | 4 | 1 | 6 |
| M.S. present | 0 | 0 | 0 | 0 |

If the comparisons in Tahle VI were restricted to the eighteen Romney Marsh soils analysed by both methods, the values would be identical. It is not possible, therefore, from these data to assess the comparative values of the two methods.

Steenbjerg attached considerable importance to the value of the term $q$ which is based on the rate of extraction of the salt-soluble manganese. For the two alkaline soils (samples 9 and 12 in the Appendix), which contained little soluble manganese but carried peas free from disease, there was no evidence that the manganese was particularly readily soluble. Their $q$ values were as high as those for several of the alkaline soils on which the peas were affected. There seems therefore little to be gained by employing the more laborious Steenbjerg series of analyses

in place of a single salt extraction made under much more rigidly controlled conditions. Comparison of the 0·05$N$ and the 1$N$ calcium nitrate extracts also failed to account for the anomalous position of samples 9, 12 and 13.

It must be admitted that there is at present no satisfactory method for dividing the salt-soluble manganese into fractions likely to be more or less rapidly available to plants. As has already been pointed out, the relationships between the different forms of active manganese in the soil are far more complicated than those for other cations. Indeed it is doubtful whether the term "exchangeable manganese" is properly applicable to soils such as those from Romney Marsh, which contain free oxides of manganese. Throughout this paper therefore the expression "readily soluble manganese" has been used instead.

Acetic acid and citric acid extracted much larger amounts of manganese than did the salt solutions (Table VII). The large amounts dissolved by citric acid are evidently derived from manganese oxides by reduction, and thus citric acid should not be used in attempts to determine readily soluble manganese. Neither of the acids served to group the soils according to the distribution of Marsh Spot.

Table VII. *Comparison of salt-soluble and acid-soluble manganese of Romney Marsh soils*

| | Soils without Marsh Spot mg. Mn % by | | | | Soils with Marsh Spot mg. Mn % by | | |
|---|---|---|---|---|---|---|---|
| Soil | 1$N$ $Ca(NO_3)_2$ | $M/2$ acetic acid | 1% citric acid | Soil | 1$N$ $Ca(NO_3)_2$ | $M/2$ acetic acid | 1% citric acid |
| 4 | 3·8 | — | 7·4 | 23 | 1·9 | 5·2 | — |
| 5 | 5·5 | 5·5 | — | 25 | 1·6 | 11·3 | — |
| 11 | 2·3 | 6·0 | 18·8 | 29 | 2·2 | 5·5 | 10·8 |
| 13 | 0·8 | 3·4 | 10·0 | 30 | 1·6 | 6·4 | 12·0 |
| | | | | 31 | 1·5 | 3·6 | 6·0 |
| | | | | 32 | 2·0 | 12·9 | 12·0 |
| | | | | 35 | 2·5 | 9·6 | 10·8 |

Attempts to determine the active manganese dioxide by extracting with a neutral ammonium acetate solution containing hydroquinone failed to distinguish soils according to the occurrence of Marsh Spot. These results did not agree therefore with Leeper's (1935) findings for Grey Speck soils in Australia.

Marsh Spot develops late in the maturity of the plant, and it might have been expected that the soil manganese of deeper horizons would have been of importance. Analyses from subsoil horizons, however, gave similar results to those for surface samples and need not therefore be given separately.

It appears that there is at present no single criterion by which soils liable to cause Marsh Spot in peas may be recognized in advance. On some soils, as, for example, the Lincolnshire silt soils (Table V), which contain but small quantities of readily soluble manganese, the disease occurs where the amount is particularly low. These soils appear to resemble those overlimed sandy heaths on which Grey Speck disease of oats is liable to occur.

The Romney Marsh soils form a separate class, since they contain considerable amonnts of manganese oxides. On these soils Marsh Spot of peas has not been found at *p*H values below 7·0. Freedom from disease appears to require comparatively large supplies of readily soluble manganese. This suggests that the manganese oxides are unavailable to plants, and also that the oxides in some way affect the availability of that portion of the soil manganese which is salt soluble. It may be that the salt-soluble manganese is derived from the unavailable oxides by reduction during the actual extraction process. Similarly in the field available manganese may be produced from these oxides by temporary reducing conditions, by the fermentation of crop residnes and farmyard manure, or by acidifying fertilizers, such as sulphate of ammonia. The analysis of the effects of these factors is obviously much more difficult than for calcium or other exchangeable ions for which there is a reversible equilibrium between the colloid complex and the soil solution.

## Pot culture experiments

The foregoing analyses are in accordance with the view that Marsh Spot of peas depends in part on the solubility of soil manganese, and results from a preliminary series of pot culture experiments give some further support. Through insufficient replication the results are to be regarded as indicative rather than conclusive. The soils used included a light sandy soil from Warwickshire on which oats in the field developed Grey Speck, and the Lincolnshire silt soil on which peas were subject to Marsh Spot. In addition peas were grown in mixtures of sand with 5 % calcium bentonite. Some pots in each series received a dressing of manganese sulphate (60 mg. Mn to about 4 kg. soil). After harvesting, Marsh Spot was present to the extent of up to 40 % of the peas in the untreated pots, but in all cases where manganese had been added the peas were healthy, or, at most, only a few per cent were damaged. Table VIII contains the results for the percentage of seeds affected by Marsh Spot in the individual pots.

Table VIII

*Percentage of seeds affected by Marsh Spot in individual pots*

| Soils | Untreated soils | | Soils with added manganese | |
|---|---|---|---|---|
| 1. Dutch soil with Grey Speck in oats | 15 | 18 | 0 | — |
| 2. Lincolnshire soil with Marsh Spot in peas | — | — | — | — |
| 2*a*. Surface 0–6 in. | 19 | 24 | 0 | 0 |
| 2*b*. Subsoil 6–16 in. | 20 | — | 0 | 0 |
| 2*c*. Subsoil 2 ft. | 41 | 25 | 0 | 4 |
| 3. Sand + bentonite | 12 | 15 | 0 | 0 |

*Analyses of pea seeds*

Mn as parts per million of oven-dry seeds

| | Untreated soils | | Soils with added manganese sulphate |
|---|---|---|---|
| | With Marsh Spot | Without Marsh Spot | Without Marsh Spot |
| Average of four soils | 10 | 12 | 17 |
| Sand + bentonite | 14 | 17 | 80 |

Determinations of manganese in seeds with and without spots from the same pods showed only very small differences (Table VIII). Addition of manganese to the soils in which the peas were grown increased the manganese content of the seeds by about one-half, but the total content remained very small and only a very small fraction of the manganese added to the soil reached the crop. In the sand-bentonite series, however, the manganese sulphate increased the amount of manganese in the seeds fivefold. The availability of added manganese, like that of the natural soil manganese, clearly depends on a variety of soil conditions not as yet determined.

## Summary

1. From an examination of thirty-five soil samples collected in Furneaux & Glasscock's survey of pea soils of the Romney Marsh area of Kent it was shown that the Marsh Spot disease of peas is more closely related to soil reaction than to soil series or soil texture. The disease was not found on any acid soil but was present on most of the alkaline soils.

2. Most of the Romney Marsh soils contained appreciable amounts of free oxides of manganese and of salt-soluble manganese. The soils with Marsh Spot contained less salt-soluble manganese than those on which peas were free from the disease, but this relation depended essentially on the contrast between acid and alkaline soils. Within the alkaline group of Romney Marsh soils, however, analyses for salt-soluble manganese did not always distinguish between contrasted soils; three of the

five alkaline soils on which peas were free from the defect were low in salt-soluble manganese.

3. A single extraction with normal calcium nitrate proved as effective as Steenbjerg's series of leachings with normal magnesium nitrate in characterizing the soils. Dilute acids dissolved more manganese than salt solutions. Citric acid gave higher results through reducing the oxides of manganese. The acid-soluble manganese was not related to the occurrence of Marsh Spot.

4. Soils on which oats suffered from Grey Speck disease and sugar beet from "Speckled Yellows" contained little or no salt-soluble manganese. Applications of manganese sulphate, which controlled the diseases in the field, appreciably increased the salt-soluble manganese in the soils.

5. Peas grown in pot cultures in manganese-deficient soils and in a sand-bentonite mixture developed Marsh Spot. Addition of manganese sulphate increased the manganese content of the seeds, especially in sand-bentonite, and controlled the disease.

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## APPENDIX

*Manganese and occurrence of Marsh Spot*

| Soil no. | Soil series | Variety* susceptibility | Percentage of peas affected | pH | Manganese, mg. Mn per 100 g. soil, extracted by: $N$ $Mg(NO_3)_2$ | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $S$ | $q$ | $N$ $Ca(NO_3)_2$ | $0.05N$ $Ca(NO_3)_2$ | $MnO_2$ mg. per 100 g. soil |
| | | | | Romney Marsh | | | | | |
| Acid soils without Marsh Spot: | | | | | | | | | |
| 1 | NR | D | 0 | 6·8 | 1·2 | 1·5 | — | — | 10 |
| 2 | F | B | 0 | 6·1 | 3·9 | 0·2 | 5·6 | — | 100 |
| 3 | NR | A | 0 | 6·6 | 4·0 | 0·1 | 4·2 | — | 10 |
| 4 | N | B | 0 | 6·4 | 2·2 | 0·6 | 3·8 | 1·4 | >100 |
| 5 | NR | A | 0 | 5·8 | 5·3 | 0·2 | 5·0 | 4·0 | — |
| 6 | NR | C | 0 | 6·0 | — | — | 6·4 | 3·5 | 0 |
| 7 | NR | D | 0 | 6·9 | — | — | 2·1 | 1·2 | 10 |
| 8 | NR | D | 0 | 5·6 | — | — | 4·5 | 4·0 | 0 |
| Alkaline soils without Marsh Spot: | | | | | | | | | |
| 9 | N | A | 0 | 7·8 | 0·6 | 3·7 | — | — | 10 |
| 10 | B | B | 0 | 7·6 | 3·3 | 0·5 | 5·2 | — | >100 |
| 11 | N | B | 0 | 7·8 | 2·2 | 0·6 | 3·8 | 1·4 | 10 |
| 12 | NR | B | 0 | 8·0 | 0·6 | 3·9 | 0·8 | 0·5 | 10 |
| 13 | NR | C | 0 | 7·4 | — | — | 0·8 | 0·5 | 0 |
| Alkaline soils with Marsh Spot: | | | | | | | | | |
| 14 | N | B | 74 | 7·8 | 2·7 | 4·4 | — | — | 10 |
| 15 | N | A | 69 | 7·0 | 1·6 | 3·5 | — | — | 10 |
| 16 | B | B | 80 | 8·1 | 0·5 | 4·5 | — | — | 100 |
| 17 | NR | B | 25 | 7·1 | 0·7 | 3·7 | — | — | 100 |
| 18 | I | C | 57 | 7·2 | 1·7 | 1·2 | 2·5 | — | 10 |
| 19 | F | C | 33 | 8·2 | 0·6 | 4·0 | 2·3 | — | 10 |
| 20 | B | B | 43 | 7·6 | 0·5 | 6·0 | 1·3 | — | >100 |
| 21 | B | A | 17 | 8·0 | 1·0 | 4·0 | 1·5 | — | 10 |
| 22 | NR | A | 25 | 7·9 | 0·6 | 6·6 | 1·4 | — | 10 |
| 23 | I | B | 2 | 8·0 | 0·6 | 4·1 | 1·9 | 0·5 | 10 |
| 24 | B | B | 1 | 7·8 | 0·9 | 2·0 | 2·5 | 0·9 | 10 |
| 25 | NR | A | 2 | 7·8 | 0·9 | 4·1 | 1·6 | 0·4 | 10 |
| 26 | N | C | 98 | 8·0 | 1·9 | 3·3 | 2·0 | 0·2 | 100 |
| 27 | NR | A | 33 | 7·6 | 1·1 | 3·1 | 1·4 | 0·9 | 10 |
| 28 | NR | A | 19 | 7·5 | 0·5 | 4·1 | 0·8 | 0·2 | 0 |
| 29 | N | A | 42 | 7·6 | — | — | 2·2 | 0·8 | 10 |
| 30 | N | A | 19 | 7·9 | — | — | 1·6 | 0·5 | 10 |
| 31 | I | E | 63 | 7·7 | — | — | 1·5 | 0·5 | 100 |
| 32 | I | A | 5 | 7·8 | — | — | 2·0 | 0·6 | 10 |
| 33 | F | E | 31 | 7·9 | — | — | 1·0 | 0·3 | 0 |
| 34 | B | E | 38 | 7·9 | — | — | 1·6 | 0·5 | 10 |
| 35 | I | B | 3 | 8·0 | — | — | 2·5 | 0·7 | 10 |
| | | | | Kent soils outside Romney Marsh | | | | | |
| Acid and alkaline soils without Marsh Spot: | | | | | | | | | |
| 36 | Wye | E | 0 | 5·1 | 5·3 | 0·02 | — | — | >100 |
| 37 | Wye | E | 0 | 8·0 | 2·8 | 0·9 | — | — | 100 |
| 38 | Hartley | E | 0 | 5·8 | 9·8 | 0·03 | 21·0 | — | >100 |
| 39 | Wye | E | 0 | 7·9 | 2·3 | 1·0 | 7·0 | — | 10 |
| 40 | Hildenborough | A | 0 | 7·6 | — | — | 6·2 | — | — |
| 41 | Coldharbour | D | 0 | 7·8 | — | — | 3·8 | — | — |
| 42 | Chart | B | 0 | 7·8 | — | — | 3·6 | — | — |
| 43 | Chart | C | 0 | 8·0 | — | — | 3·8 | — | — |
| 44 | Chart | A | 0 | 7·9 | — | — | 3·8 | — | — |
| Alkaline soil with Marsh Spot: | | | | | | | | | |
| 45 | Wye | A–E | 0–36 | 8·0 | 1·6 | 2·3 | 1·3 | 0·2 | >100 |

* In trials at Wye the percentages affected by Marsh Spot were: A, none; B, 1–3%; C, 3–10%; D, 10–20% E, over 20%.

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# AN ACCURATE WET-COMBUSTION METHOD FOR THE DETERMINATION OF CARBON IN SOILS

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(With Three Text-figures)

## Introduction

The standard method for the determination of carbon in soils is the dry-combustion method in which the soil is burnt in an atmosphere of oxygen in the presence of various oxidizing agents e.g. cupric oxide. The carbon dioxide produced is absorbed and weighed. This method has been in use for some years in the Agricultural Chemistry laboratory of the University of Pretoria, and, when due care has been taken, has yielded accurate results. There are, however, certain difficulties inherent in the method. It is tedious, and some practice is required with the technique of the method before accurate results are obtained. Difficulty has also been experienced with the absorption train. In order to ensure complete elimination of the various secondary products of combustion of the soil (e.g. oxides of nitrogen), numerous absorbents must be introduced into the train and combustion must proceed slowly. Attempts to "speed up" determinations have resulted in experimental errors.

From time to time various wet-combustion methods have been suggested, as alternative to the dry-combustion method, but none of these has hitherto proved satisfactory. Warington & Peake (1880) used sulphuric acid and potassium bichromate and obtained lower results than with the dry-combustion method. Hall & Muller (1906) concluded that the error was due to incomplete oxidation, and passed the products of combustion over heated copper oxide.

Ames & Gaither (1914) compared (*a*) combustion with concentrated chromic acid, (*b*) combustion with dilute chromic acid, and (*c*) combustion with concentrated chromic acid, passing the products of combustion over heated copper oxide. They came to the conclusion that concentrated chromic acid gave higher results than dilute chromic acid, but that passing the products of combustion over heated copper oxide produced no appreciable difference in results. This was probably because

the losses due to the escape of volatile organic compounds were within the limits of experimental error.

Schollenberger (1927) suggested a rapid method whereby the organic carbon of the soil is oxidized with chromic acid and sulphuric acid at 175° C., and the residual chromic acid determined by titration.

Robinson *et al.* (1929) measured the sulphur dioxide produced in the digestion of soil with concentrated sulphuric acid, and found the carbon content of the soil to be approximately proportional to the sulphur dioxide evolved.

Walkley (1935) recently compared various methods, and found the results obtained by the wet-combustion methods to be low, taking the dry-combustion method as the standard.

It is generally accepted that wet-combustion methods give only approximate results, the approximation varying in degree in different soils. These methods have, however, the advantage that they are comparatively simple and rapid, and they are therefore extensively used.

The desirability of a method which combines the advantages of wet combustion with the accuracy of dry combustion is obvious. In an attempt to devise such a method the work here reported was undertaken.

In the method here described the soil is digested with sulphuric acid and potassium bichromate, the products of combustion passed through a heated combustion tube, and the carbon dioxide absorbed in barium hydroxide. Digestion and absorption take place in a closed system which allows neither the escape nor entrance of carbon dioxide.

The results obtained agree well with results by the dry-combustion method, and with theoretical values for pure organic compounds. Agreement between duplicate determinations is excellent. As compared with dry combustion, this method reduces experimental error, as the long train is eliminated, and a larger quantity of soil may be used for each determination. It is, moreover, more rapid and simpler than the dry-combustion method.

## Apparatus

The apparatus is shown in Fig. 1. A 300 or 500 ml. Kjeldahl flask (*A*) is fitted with a 50 ml. separating funnel (*B*) and a double-surface or spiral water-cooled condenser (*C*). The separating funnel is connected to an air-scrubber consisting of a *U*-tube (*X*) containing ascarite, a wash-bottle (*Y*) containing concentrated $H_2SO_4$ and a soda-lime tower (*Z*). The $H_2SO_4$ indicates the rate of flow of air into the system.

The Kjeldahl is arranged on a slant to avoid charring the stopper.

The gases pass from the Kjeldahl flask through the condenser, then through a combustion tube ($D$). This tube is filled with coarse sand and heated to a temperature of 900–1000° C. The sand retards the free flow of gas and ensures complete oxidation of volatile organic compounds.

The combustion tube is connected by means of a glass tap ($L$) to a pressure gauge ($E$). The pressure gauge is very simply made of glass tubing ¼ in. in diameter. A 9 in. length of tubing is bent to form a **V**, with two horizontal arms. A second length of tubing of about 18 in. is sealed in at the base of the **V**, and then bent to form a narrow **U** with the free arm of the **U** a few inches longer than the other arm. The top of the longer arm is widened to form a little cup, and the gauge is filled with mercury to the level indicated in the diagram.

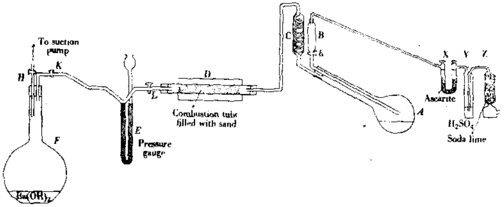


Fig. 1.

A strong 2 litre boiling flask is used for the absorption of the carbon dioxide. This flask ($F$) is fitted with a two-hole stopper through which pass two glass taps ($H$ and $K$). $K$ has a right-angle bend and is connected to the arm of the pressure gauge by means of rubber tubing about 6 in. in length which allows the absorption flask to be freely shaken. The second tap ($H$) is connected to a suction pump.

*Apparatus for introducing* $Ba(OH)_2$ *solution into the absorption flask*

A 10 litre aspirator bottle containing saturated $Ba(OH)_2$ solution is connected to a 50 c.c. automatic pipette. The automatic pipette is fitted with glass tubing, as indicated in Fig. 2. It is connected to a 3 litre flask containing $CO_2$ free distilled water. The water is shut off from the pipette by means of a screw clip ($O$).

The pipette is protected from atmospheric carbon dioxide by means of a soda-lime tube fitted with a glass tap ($M$). The aspirator bottle and distilled water are also protected with soda-lime tubes.

*Reagents*

(1) Saturated $Ba(OH)_2$ solution. (2) Concentrated $H_2SO_4$. (3) Powdered $K_2Cr_2O_7$. (4) Hydrogen peroxide (20 vol.). (5) 0·2*N* HCl. (6) 0·2*N* NaOH. (7) Indicators: Thymolphthalein; methyl red.

METHOD

The combustion tube is heated to redness (900–1000° C.). 6 g. of soil are weighed into the Kjeldahl flask and 10 c.c. of $H_2O$ added with a

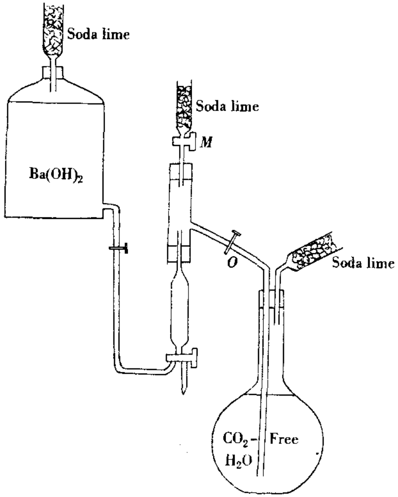


Fig. 2. Apparatus for introducing Ba $(OH)_2$ solution into absorption flask.

pipette. When the soil is thoroughly moistened, 5–8 g. of powdered $K_2Cr_2O_7$ is added. The Kjeldahl is then connected to the apparatus. 1–2 c.c. of thymolphthalein is introduced into the absorption flask, after which it is also connected. All the taps are opened and gentle suction is applied for 2 min. in order to draw $CO_2$ free air into the system. Taps *H*, *K*, *L* and *b* are then closed, and the absorption flask is disconnected.

Tap *K* of the absorption flask is now connected to the suction pump, and the flask is evacuated. Tap *H* is connected to the automatic pipette by means of rubber tubing and, with tap *M* open, the requisite amount of $Ba(OH)_2$ solution (50–250 c.c., depending on the amount of carbon in the sample) is run into the flask. Tap *M* is now closed and screw-clip *O* opened, so that distilled water is drawn into the pipette and run into the flask. This washes the stem of tap *H* free of $Ba(OH)_2$ solution, and prevents carbonate from forming here. The suction on the flask should be continued after introducing the $Ba(OH)_2$ solution, to ensure that it is completely evacuated. After evacuation the flask is again connected to the apparatus. Tap *L* is opened. Then 40 c.c. of conc. $H_2SO_4$ are introduced into the separating funnel *B*. The latter is again connected to the air-scrubber, and the $H_2SO_4$ is run into the Kjeldahl flask, a few drops at a time. The reaction between the water in the Kjeldahl and the $H_2SO_4$ introduced will cause an increase in pressure which will be indicated by the gauge.

Tap *K* is opened slightly to allow gas to pass into the absorption flask. This tap is shut when the pressure in the system is reduced to slightly below atmospheric pressure. This operation is repeated, *b* and *K* being alternately manipulated until all the $H_2SO_4$ has been introduced into the Kjeldahl.

The Kjeldahl is now heated over a bunsen burner, gently at first. As the pressure increases, *K* should be manipulated, allowing gas to pass into the absorption flask when necessary. During the digestion the pressure gauge should be watched, the stopper *K* being carefully manipulated so that the pressure in the system fluctuates between atmospheric and 2–3 in. above atmospheric pressure.

Digestion should be continued for 15 min. The flame should be small, as there is a tendency for the contents of the Kjeldahl to froth.

After 15 min. digestion the flame is removed, tap *b* is opened and, by opening *K*, at first slightly, then completely, $CO_2$-free air is sucked through the system into the absorption flask.

The rate at which air enters should be such that the absorption flask reaches atmospheric pressure in 5–10 min. Before disconnecting the absorption flask it should be ascertained that the $CO_2$ in the flask has been completely absorbed by the $Ba(OH)_2$. This is done by closing *K*, shaking the flask for a few seconds and then opening *K*. If $CO_2$ is still present, air will be seen to enter the system through *Y*, in which case shaking should be continued till air ceases to enter.

A sufficiency of $Ba(OH)_2$ in the absorption flask is indicated by the

blue colour of the thymolphthalein. Most methods of absorption of $CO_2$ have consisted of passing the gases evolved through an absorbent and allowing the unabsorbed gases to escape. Preliminary determination carried out indicate that, unless the digestion is exceedingly slow, this method of absorption allows the escape of $CO_2$. It is possible that the low results obtained by methods previously described have been due to faulty absorption. The method here described provides for the absorption of $CO_2$ in a closed system, so that the escape of $CO_2$ is impossible and the absorption must be complete.

## Titration

The use of thymolphthalein in place of phenolphthalein has been discussed by Schollenberger (1928). Thymolphthalein gives a more definite end-point for this titration, and is therefore a more satisfactory indicator to use.

The absorption flask is disconnected and $0{\cdot}2\,N$ HCl is run into the flask from a burette until the excess $Ba(OH)_2$ is exactly neutralized, as indicated by the disappearance of the blue colour.

The absorption flask now contains, in addition to the carbonate, some sulphate and sulphite which distils over in the course of digestion. The sulphites will interfere with the titration of the carbonate, and must therefore be oxidized before the carbonate can be determined.

This is done by adding 5 c.c. of $H_2O_2$ to the contents of the flask and boiling for about 2 min. It should be noted that the free $Ba(OH)_2$ should be neutralized with HCl before adding the $H_2O_2$, as the addition of $H_2O_2$ to $Ba(OH)_2$ will cause the precipitation of $BaO_2$ which interferes with the titration.

After the oxidation, methyl red is added as indicator, a measured excess of $0{\cdot}2\,N$ HCl is run into the flask and the contents of the flask boiled to expel $CO_2$; the excess of HCl is subsequently determined by titration with $0{\cdot}2\,N$ NaOH. The HCl absorbed gives a measure of the carbon in the sample. 1 c.c. of $0{\cdot}2\,N$ HCl is equivalent to 0·0012 g. carbon.

A blank determination should be carried out with the reagents.

## Results

Determinations were carried out on pure organic compounds, samples of coal and a variety of soils. The results are given in the following tables.

Table I. *Pure organic compounds*

Carbon per cent

| | Theoretical | Wet-combustion method: As described in text | Wet-combustion method: Without combustion tube |
|---|---|---|---|
| Urea | 19·98 | 19·98 | 19·98 |
| Amino-benzoic acid | 61·29 | 61·30 | 60·3—60·19 |
| *Benzoic acid* | *68·79* | *68·75* | *66·1—67·8* |
| Succinic acid | 40·66 | 40·63 | — |
| Oxalic acid | 17·91 | 17·90 | — |

Table II. *Coal*

Carbon per cent

| Sample no. | Dry combustion* | Wet combustion |
|---|---|---|
| 1 | 76·1 | 76·2 |
| 2 | 80·0 | 79·1 |
| 3 | 82·1 | 80·0 |
| 4 | 75·6 | 73·9 |

Table III. *Soils*

Carbon per cent

| | | Dry combustion* | Wet combustion: As described in text | Wet combustion: Without combustion tube | Wet combustion: Direct use of 80% $H_2SO_4$ on dry sample |
|---|---|---|---|---|---|
| 1. | Brown loam | 0·947 | 0·949 | 0·948 | 0·946 |
| 2. | Black peat | 25·700 | 25·840 | 25·550 | 25·990 |
| 3. | Black loam (*ex* George) | 9·670 | 9·630 | 9·470 | 9·580 |
| 4. | Black soil (residue of weathered coal) | 11·050 | 10·980 | 10·870 | 10·310 |
| 5. | Black clay soil | 3·470 | 3·410 | 3·350 | 2·510 |
| *6.* | *Brown loam* | *1·270* | *1·250* | *1·220* | *1·150* |
| 7. | Alkali soil (*ex* Sunday's river) | 0·850 | 0·860 | 0·860 | 0·860 |
| 8. | Black peat | 11·470 | 11·370 | 11·070 | 11·280 |
| 9. | Alkali clay | 1·370 | 1·380 | 1·350 | 1·360 |
| 10. | Alkali clay loam | 2·130 | 2·155 | 2·110 | 2·087 |
| 11. | Loam from arid region | 1·860 | 1·865 | 1·786 | 1·704 |
| 12. | Loam from semi-arid region | 0·665 | 0·666 | 0·666 | 0·665 |
| 13. | Clay loam from semi-arid region | 5·150 | 5·170 | 5·110 | 5·140 |
| 14. | Heavy loam | 0·596 | 0·595 | 0·596 | 0·575 |
| 15. | Clay from subtropical region | 0·931 | 0·937 | 0·925 | 0·872 |
| 16. | Clay from subtropical region | 1·320 | 1·310 | 1·290 | 1·300 |
| 17. | Clay sub-humid region | 1·113 | 1·115 | 1·090 | 0·893 |
| 18. | Loam semi-arid region | 2·126 | 2·070 | 2·020 | 2·040 |
| 19. | Clay loam fairly alkaline | 0·726 | 0·732 | 0·686 | 0·698 |
| 20. | Heavy loam | 0·775 | 0·766 | 0·740 | 0·701 |
| 21. | Clay loam | 0·620 | 0·646 | 0·629 | 0·612 |
| 22. | Clay loam fairly alkaline | 0·980 | 0·952 | 0·951 | 0·891 |
| 23. | Clay very alkaline | 0·869 | 0·859 | 0·82 | 0·807 |

* Figures of dry combustion were kindly supplied by Mr L. P. van Wyk of this Laboratory.

DISCUSSION OF METHOD.

*Resistant carbon.* In the case of the resistant carbon of coal it was found necessary to use selenium as a catalyst, and continue digestion for ½ hr. to ensure complete oxidation of all the carbon. Approximately 10 g. of the mixture containing 2 parts of selenium to 100 parts of $K_2SO_4$ were used in addition to the powdered $K_2Cr_2O_7$ in digesting the sample.

*The use of the combustion tube.* Determinations were carried out to ascertain whether the heated combustion tube was necessary. It appears that small but appreciable amounts of carbon escape from the Kjeldahl in the form of volatile compounds. It is interesting to note that, in the case of soils, remarkably good agreement was obtained between duplicate determinations even when the combustion tube was not used. Differences between duplicate determinations did in no case exceed 6 parts per 1000. Apparently the same fraction of the organic matter consistently passes over incompletely oxidized.

It appears that where absolutely accurate results are required, the combustion tube should be used; at other times it may be omitted.

*Preliminary moistening of the soil with water.* In the case of pure organic compounds and coal, accurate results were obtained when $H_2SO_4$ was added to the dry sample. When, however, soil was analysed, it was found that, with few exceptions, the results obtained were low compared with the dry-combustion method. Duplicate determinations, too, did not show good agreement.

Bal (1925), in determining nitrogen in soil by the ordinary Kjeldahl method, obtained higher results in the case of certain heavy soils when water was added before the $H_2SO_4$ employed for the digestion. He suggested that the low results by the ordinary Kjeldahl procedure were due to the presence of some cementing substances soluble in water but not in conc. $H_2SO_4$. Walkley (1935) confirmed this finding on some alkaline soils, and offered the alternative explanation that the heavy alkaline soils failed to disperse in the non-polar strong $H_2SO_4$. The results given in Table III indicate that moistening of soil is necessary in most cases. The effect of soaking the soil for various periods was tried, and it was found that with 12 g. of soil and 10 c.c. of water, thorough wetting of the soil particles was as effective as a longer period of soaking. Periods of soaking ranging from 2 min. to 24 hr. produced no difference in results.

It is possible that the incomplete digestion of carbon which occurs when the soil is not first moistened is due to the reactive silicic acid of the

soil, which forms a gelatinous coat around the soil particles and prevents the attack of the organic matter by the acid.

*Time of digestion.* Periods of digestion ranging from 10 to 60 min. were tried. In all soils a period of 15 min. gave complete oxidation of carbon. Longer periods showed no increase in results. In the case of coal, as has already been noted, it was necessary to continue digestion for 30 min.

*Effect of different strengths of acid.* The effect of different strengths of $H_2SO_4$ ranging from conc. $H_2SO_4$ to 80% (by volume) $H_2SO_4$ was tried on coal. In the presence of catalysts (selenium and potassium sulphate) complete digestion of carbon was obtained with all strengths of acid within these limits.

*Effect of different concentrations of potassium bichromate.* Quantities of $K_2Cr_2O_7$ between 5 and 10 g. give complete oxidation of the carbon. Where less $K_2Cr_2O_7$ is used, it sometimes becomes necessary to increase the time of digestion. It is inadvisable to use more than 8 g. of $K_2Cr_2O_7$, as frothing tends to become more troublesome with increased quantities of this salt.

*Use of catalysts.* Determinations were carried out on twenty-five soils, with and without the above-mentioned catalyst, viz. selenium and $K_2SO_4$. In no case did the catalyst make any difference to the result.

### APPLICATION OF THE METHOD TO SOILS RICH IN CHLORIDES.

With soils containing a high percentage of chlorides, the potassium bichromate and sulphuric acid react with the chloride, liberating free chlorine. This passes over into the absorption flask and reacts with the $Ba(OH)_2$ precipitating $BaO_2$, which interferes with the titration of the carbonate. Moreover, if a sufficient quantity of chlorine is liberated, this bleaches the thymolphthalein and titration is impossible.

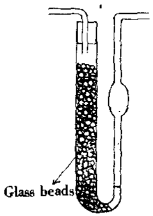

Fig. 3.
Absorption tower.

In order to eliminate the chlorine, an absorption tower as shown in Fig. 3 is introduced into the system between the condenser and the combustion tube. 5 c.c. of a concentrated solution of sodium bisulphite is introduced into the absorption tower. The sodium bisulphite acts as an effective absorbent of the chlorine. The solution should be renewed after about ten determinations. Where the sodium bisulphite amount of $SO_2$ which passes into the $Ba(OH)_2$ is naturally much is used, the increased. In order to oxidize the sulphite to sulphate, 10 c.c. of hydrogen peroxide should be used instead of 5 c.c., as is used normally.

## Summary and conclusions

1. Inaccurate results previously obtained with wet-combustion methods for the determination of carbon in soils have probably been due to: (*a*) incomplete oxidation of carbon; (*b*) incomplete absorption of carbon dioxide. The method described herein ensures complete oxidation and complete absorption. The oxidizing agent employed consists of a mixture of sulphuric acid and potassium bichromate; the absorption takes place in a closed system.

2. When highly accurate results are required, a combustion tube is a necessary adjunct to the wet-combustion method, as appreciable amounts of carbon escape complete oxidation in reaction flask.

3. The soil should be moistened with water before adding the oxidizing mixture, to avoid the formation of complexes, which may protect part of the carbon from oxidation by the acid.

4. Within limits, the concentrations of sulphuric acid and potassium bichromate do not affect the results.

5. While it is necessary to use catalysts (selenium and potassium sulphate) for the complete oxidation of the resistant carbon of coal, catalysts were found to be unnecessary for the determination of carbon in soils.

6. For soils rich in chloride, it is necessary to absorb the chlorine evolved by means of sodium bisulphite solution.

## Acknowledgements

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# THE EFFECT OF NITROGENOUS FERTILIZERS ON THE CALCIUM STATUS OF SOIL

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Most of the work published on the "physiological reaction" of nitrogenous fertilizers has been based on the results of pot and laboratory experiments. Apart from information on the effects of ammonium sulphate and sodium nitrate there appears to be a dearth of results based on field experiments, particularly with the newer types of fertilizer materials.

The aim of the experiment described in this paper was to study the effect of nine nitrogenous fertilizers on the rate of loss of exchangeable calcium from the soil and on crop yields.

## Soil

The mechanical composition of the soil is given in Table I. The soil, which is derived from London Clay, contained no reserve of calcium carbonate.

Table I. *Percentage mechanical composition of soil (oven-dry basis)*

| | 0–9 in. | 9–18 in. |
|---|---|---|
| Coarse sand | 33·6 | 31·4 |
| Fine sand | 34·5 | 32·9 |
| Silt | 12·3 | 12·5 |
| Clay | 13·5 | 20·5 |
| Moisture | 6·9 | 1·8 |
| Loss on solution | 1·5 | 1·5 |
| Loss on ignition | 4·5 | 3·7 |

## Rainfall

The rainfall during the course of the experiment was 23·65 in. in 1932, 19·84 in. in 1933, 23·43 in. in 1934 and 30·52 in. in 1935.

## Treatments

The following treatments were compared: (i) no nitrogen, (ii) ammonium sulphate, (iii) ammonium nitrate, (iv) calcium nitrate, (v) calcium cyanamide, (vi) nitrate of soda, (vii) nitrochalk, (viii) urea, (ix) monoammonium phosphate, and (x) diammonium phosphate.

The materials used were of ordinary commercial grade. Nitrochalk consists of approximately 50% ammonium nitrate and 50% calcium carbonate.

## Layout

Each treatment was replicated three times, the plots being arranged in three random blocks; individual plots were 0·025 acres.

## Cropping

It was originally planned to continue the experiment over several rotations, but this was not possible and the experiment was stopped at the end of the first cycle. The crops taken and fertilizers applied were as follows:

| | | Sown | Harvested | Lb. N applied per acre | Date of applying N |
|---|---|---|---|---|---|
| 1932 | Mangolds | 18 May | Nov. | 69 | 8 May* |
| 1933 | Oats | 31 Mar. | 25 July | 23 | 10 Apr. |
| 1934 | Seeds (20 lb. perennial ryegrass, 4 lb. red clover) | May | 4 June | 23 | 10 Apr. |
| | | | 17 Aug. | 23 | 14 June |
| 1935 | Wheat | 10 Oct. (1934) | 1 Aug. | 23 | 22 Mar. |

* Except calcium cyanamide which was applied on 27 April.

The area had been heavily dressed with phosphates and potash in previous years and a basal dressing of 5 cwt. superphosphate (14% w.s. $P_2O_5$) and 200 lb. muriate of potash per acre were applied to *all* plots on 8 May 1932. No phosphates or potash were applied in 1933, 1934 or 1935, but there is strong reason to suppose that the supply in the soil was adequate. It should be noted that treatments 9 and 10 received extra phosphate each year in addition to the dressing of superphosphate in 1932.

## Crop data

The crop results are given in Table II. Composite samples per treatment were analysed for calcium and nitrogen.

In 1932, the leaves of the mangold crop were ploughed in, the leaves on each plot being kept on that plot. Owing to an unfortunate error, sodium chlorate was applied to the sodium nitrate plots in 1935 and yields of wheat for this treatment are, therefore, not available.

The results show a fairly good response to nitrogen each year but in no case was there any significant difference between the effects of the various nitrogenous fertilizers.

The average recovery of added nitrogen in those parts of the crops removed was 40·5%; if allowance be made for the nitrogen in the

mangold tops in 1932, the recovery was probably about 50%. These recovery figures are, of course, only approximate since they take no account of fixation of nitrogen by legumes (1934) or of nitrogen in the roots.

Table II. *Crop results*

| | 1932. Mangolds (roots only) | | | 1933. Oats | | | |
|---|---|---|---|---|---|---|---|
| | Fresh tons | N lb. | CaO lb. | Grain cwt. | Straw cwt. | N lb. | CaO lb. |
| 1. No nitrogen | 21·72 | 68·8 | 11·76 | 14·90 | 22·90 | 34·7 | 9·01 |
| 2. Sulphate of ammonia | 29·01 | 103·3 | 13·90 | 19·62 | 29·47 | 46·8 | 11·74 |
| 3. Ammonium nitrate | 30·23 | 122·2 | 17·00 | 18·59 | 27·98 | 44·9 | 12·24 |
| 4. Calcium nitrate | 29·30 | 104·4 | 14·04 | 18·99 | 29·42 | 46·4 | 11·72 |
| 5. Calcium cyanamide | 28·00 | 89·8 | 15·17 | 19·14 | 26·98 | 44·9 | 11·14 |
| 6. Nitrate of soda | 29·74 | 93·6 | 11·15 | 17·71 | 27·70 | 40·2 | 10·73 |
| 7. Nitrochalk | 26·60 | 100·3 | 13·30 | 18·62 | 28·74 | 42·7 | 11·26 |
| 8. Urea | 27·37 | 104·9 | 12·54 | 19·41 | 29·24 | 45·5 | 11·42 |
| 9. Monoammonium phosphate | 27·37 | 99·2 | 12·54 | 19·80 | 30·84 | 45·3 | 12·07 |
| 10. Diammonium phosphate | 27·41 | 97·1 | 10·28 | 17·39 | 26·91 | 40·2 | 10·75 |
| Standard error | 1·58 | — | — | 1·133 | 1·355 | — | — |

| | 1934. Seeds | | | 1935. Wheat | | | |
|---|---|---|---|---|---|---|---|
| | Dry matter cwt. | N lb. | CaO lb. | Grain cwt. | Straw cwt. | N lb. | CaO lb. |
| 1. No nitrogen | 33·0 | 44·8 | 21·9 | 17·09 | 24·96 | 35·8 | 6·38 |
| 2. Sulphate of ammonia | 44·5 | 55·4 | 28·5 | 21·52 | 31·54 | 45·0 | 8·01 |
| 3. Ammonium nitrate | 43·6 | 53·2 | 29·4 | 19·94 | 30·29 | 45·1 | 8·97 |
| 4. Calcium nitrate | 46·8 | 59·5 | 33·2 | 21·07 | 31·76 | 48·0 | 9·79 |
| 5. Calcium cyanamide | 43·3 | 54·7 | 28·7 | 21·65 | 32·19 | 48·0 | 9·25 |
| 6. Nitrate of soda | 48·6 | 62·8 | 30·3 | — | — | — | — |
| 7. Nitrochalk | 46·4 | 55·2 | 31·9 | 21·15 | 31·85 | 44·2 | 8·78 |
| 8. Urea | 45·3 | 58·4 | 28·7 | 20·77 | 30·42 | 43·7 | 8·74 |
| 9. Monoammonium phosphate | 44·7 | 56·9 | 27·9 | 23·01 | 35·13 | 47·8 | 10·44 |
| 10. Diammonium phosphate | 45·1 | 58·7 | 27·7 | 20·94 | 31·58 | 44·6 | 9·28 |
| Standard error | 2·10 | — | — | 0·928 | 1·64 | — | — |

## SOIL DATA

Samples of soil were taken on 27 April 1932 and 30 March 1936, i.e. at the same stage in the rotation and when soil density would be expected to be the same. Thirty borings were taken per plot to a depth of 9 in. with a cylindrical borer 1 in. in diameter. The soil samples were air-dried and ground. Exchangeable CaO was determined per plot by extraction with $NNH_4Cl$ to 1 litre, precipitation as oxalate and titration with $KMnO_4$ in the presence of $H_2SO_4$.

In order to save space, *p*H figures are not quoted. Although the variation in *p*H between the plots was small (maximum variation 5·6–6·3), there is a high positive correlation between *p*H and exchangeable calcium ($r = +0{\cdot}7181$).

The exchangeable calcium data are given in Table III. Since there is no apparent relation between level of exchangeable calcium and loss of exchangeable calcium, the differences in exchangeable CaO (1936–1932) were analysed statistically. It should be remembered that the 1936 figure for the nitrate of soda plots is subject to some doubt since these plots received sodium chlorate instead of sodium nitrate in 1935.

Table III. *Exchangeable* CaO: *percentage of air-dry soil* (0–9 *in.*)

| | 1932 | 1936 | Loss | Effect of fertilizers |
|---|---|---|---|---|
| 1. Control | 0·207 | 0·178 | 0·029 | — |
| 2. Sulphate of ammonia | 0·224 | 0·175 | 0·049 | −0·020 |
| 3. Ammonium nitrate | 0·260 | 0·224 | 0·036 | −0·007 |
| 4. Calcium nitrate | 0·226 | 0·211 | 0·015 | +0·014 |
| 5. Calcium cyanamide | 0·214 | 0·211 | 0·003 | +0·026 |
| 6. Nitrate of soda | 0·236 | (0·214) | (0·022) | (+0·007) |
| 7. Nitrochalk | 0·210 | 0·188 | 0·022 | +0·007 |
| 8. Urea | 0·216 | 0·181 | 0·035 | −0·006 |
| 9. Monoammonium phosphate | 0·210 | 0·173 | 0·037 | −0·008 |
| 10. Diammonium phosphate | 0·221 | 0·182 | 0·039 | −0·010 |
| Standard error | — | — | 0·004 | — |

In considering the soil data, the effect of the nitrogenous fertilizers on calcium content of the crops can be ignored since the extra amounts in the crops amounted to only 15–20 lb. CaO per acre over the rotation.

The normal loss of Ca from the soil (0·029 % or 5·8 cwt. exchangeable CaO per acre in 4 years) was greater than the loss caused by ammonium sulphate. This normal loss of Ca is rather greater than that found by Rice Williams (1936) in North Wales (about 0·005 % CaO per annum for a soil of the same content of exchangeable CaO).

As pointed out by Crowther & Basu (1931), the estimation of the relative effects of nitrogenous fertilizers from the results of field experiments is impossible if secondary effects, such as acidification by ammonium salts with consequent lowering of yields and of bicarbonate concentration of the drainage water, occur. Crowther & Basu add: "Any general statement on this much-discussed question must be restricted to the simplest case in which the plants absorb the same amounts of calcium and nitrogen and the drainage water removes a constant amount of bicarbonate." The present results appear to fulfil these conditions since the yields and uptake of Ca and N were substantially the same with all the nitrogenous fertilizers, and it can be assumed that, as the exchangeable calcium and *p*H only varied over a small range, the bicarbonate concentration of the drainage water was constant.

The results given in Table IV agree closely with Crowther & Basu's statement that "Under such (constant) conditions the loss of calcium

exceeds that for equivalent nitrogen added as nitrate by an amount equivalent to the excess of anions over the cations in the added fertilizer, all added nitrogen being regarded as nitrate and all carbon and phosphorus being ignored." The figures given in the last column of Table IV are calculated on the assumption that all the nitrogen was absorbed as nitric acid. It may be remarked here that the effect of nitrogenous fertilizers is dependent on their efficiency, the lower the recovery of added N in the plant, the greater the loss (or smaller the gain) of calcium.

Table IV

| | Parts $CaCO_3$ per 100 parts $(NH_4)_2SO_4$ or equivalent N | Mol. CaO per 2 *N* | |
|---|---|---|---|
| | | Actual | Calculated (N 50% effective) |
| Ammonium sulphate | −102 | −1·40 | −1·5 |
| Ammonium nitrate | − 36 | −0·49 | −0·5 |
| Calcium nitrate | + 71 | +0·98 | +0·5 |
| Calcium cyanamide | +133 | +1·81 | +1·5* |
| Nitrate of soda | (+ 36) | (+0·49) | +0·5 |
| Nitrochalk | + 36 | +0·49 | +0·5 |
| Urea | − 31 | −0·42 | −0·5 |
| Monoammonium phosphate | − 41 | −0·56 | −0·5 |
| Diammonium phosphate | − 51 | −0·70 | −0·5 |

* For pure $CaCN_2$.

It is interesting to note that the effects of mono- and diammonium phosphates were the same as those of ammonium nitrate and urea. This agrees with Crowther and Basu's (1931) ideas but conflicts with Pierre's (1933) estimates of "equivalent acidity". The added phosphorus was probably precipitated as a phosphate of calcium, and while this calcium is not removed from the soil it is removed from the colloidal complex of clay and organic matter. It would, therefore, appear that the calcium of such precipitated phosphates of calcium remains exchangeable and, hence, available to plants.

Since mono- and diammonium phosphates caused less loss of calcium than did ammonium sulphate it is clear that fertilizers based on ammonium phosphates cause less loss of calcium than mixtures of ammonium sulphate and snperphosphate.

## Summary

The results of an experiment, conducted over one cycle of a four-course rotation to study the effects of nine nitrogenons fertilizers on crops and soil, are described.

All the crops gave fairly good responses to nitrogen but in no case

was there a significant difference between the effects of the various nitrogenous fertilizers. The "recovery" of added nitrogen was about 40–50 % with all the fertilizers.

Figures are given for the changes in exchangeable CaO content of the soil. The results confirm Crowther and Basu's deduction that "the loss of calcium exceeds that for equivalent nitrogen added as nitrate by an amount equivalent to the excess of anions over the cations in the added fertilizer, all added nitrogen being regarded as nitrate and all carbon and phosphates being ignored".

The fact that phosphate should be ignored is shown by results for mono- and diammonium phosphates. Precipitation of phosphates in the soil almost certainly involves removal of calcium from the exchange complex but the calcium in such precipitated phosphates is exchangeable.

Since phosphate has no effect on calcium status, it is clear that fertilizers based on ammonium phosphates remove less calcium from the soil than do mixtures based on ammonium sulphate and superphosphate.

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# THE COMPOSITION OF DIFFERENT KINDS OF SILAGE

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The results of a detailed and comprehensive examination of a large number of samples of silage were reported recently by Watson & Ferguson (1937). They discussed the methods of analysis, and, for comparative purposes, subdivided the groups of silage, made by different processes, according to the *p*H values. They were able to demonstrate that the acidity of the material was of outstanding importance in determining the extent and nature of protein breakdown and the relative amounts of the organic acids formed. The present paper contains a summary of tbe analytical data, collected during the last four or five years, for about 100 samples of different types of silage examined, partly for advisory purposes and partly in connexion with a study of the A.I.V. process. As will be seen from the tables, there is a very close agreement in the majority of cases with the results given by Watson & Ferguson (1937), but there are also some interesting differences due, in all probability, to the different conditions under which the silages were made.

## Material examined

The different samples are grouped in Table I, according to the nature of the original material and the process of ensiling, and subdivided according to the degree of acidity. Figures for dry matter and crude protein and those obtained in the steam distillation of the aqueous extracts are also given in Table I.

Included in the table, are a few samples of grass mixtures and legume mixtures, which were taken from crops about to be ensiled, and were examined in the same manner as the silages, in order to obtain some idea of the composition of the fresh foodstuffs. Thirty-nine samples of silage prepared by the A.I.V. process were analysed. Group 3 consists of samples which were taken at intervals of about a week, commencing November, from different points of a wood-lined pit silo, as the silage was being used for feeding purposes. The original crop was a mixture

of beans, peas and tares cut in mid-July (see group 2). A rye grass-clover aftermath, cut at the beginning of September, was used to make the silage described in group 4. The material was treated with acid and packed in a series of six barrels, which were opened in succession at intervals of 14 days, beginning at the end of September. Group 5 consists of samples of silage from a similar experiment which was affected somewhat by excessive rainfall and inadequate drainage of the barrels; and group 6 comprises a series of early samples, which were taken when the A.I.V. process was first tried, and which were not regarded as entirely satisfactory.

It is a fairly common thing in certain parts of this country to attempt to preserve grass or an aftermath, when conditions for haymaking are bad, by compacting the material in an unlined pit or trench about 4 ft. deep, or in small stacks. In both cases, especially the latter, there is usually considerable wastage at the sides, but the silage from the centre is often quite satisfactory, and samples of such material are included in groups 7–11. Groups 12*a* and 12*b* are samples of stack silages which were made respectively from oats, peas and beans and oats and tares. The samples in groups 13 and 14 were particularly interesting inasmuch as they all came from a large tower silo—oats, bean, tare mixture—in which existed at the same time silage from the 1931, 1933 and 1934 crops on top of each other. The 1931 silage, at the bottom of the tower, had not such a good composition as the other two which were very similar in their principal properties.

Groups 15 and 16 are silages made from grass mixtures with the addition of molasses. The remainder of the samples have been added to illustrate the type of result which may be obtained when the *p*H value is above what has come to be commonly regarded as typical of good silage.

## Methods of analysis

The samples were normally taken by cutting out blocks of the silage with a hay knife from about a foot below the surface. Immediately on arrival in the laboratory, or after storage overnight in a refrigerator, the sample was chopped up and thoroughly mixed, and separate samples were taken for the preparation of the aqueous extract and the determination of the dry matter.

*Dry matter and crude protein.* A large sample was dried in an electric oven reaching a maximum temperature of 100° C. The volatile constituents were not estimated in the dried sample, so that no correction

could be made in the dry-matter figures, which are consequently too low. Watson & Ferguson (1937) found that about 77 % of the volatile acids in the fresh material was lost on drying, in the majority of their samples, and about 30–60 % of the volatile bases. An examination of the data in Tables I and II shows that the dry-matter figures are, therefore, too low by about 0·15–1·2 %, according to the amounts of volatile acid and base present. This in turn affects, but to a much smaller extent, the values for acids and nitrogen compounds, estimated in the aqueous extract, which should be slightly lower. The crude protein was estimated by determining the nitrogen in the dried material by the Kjeldahl method and multiplying by the factor 6·25. The figures for crude protein are, of course, too low, on account of the partial loss of volatile bases during drying. The error in the nitrogen figures has also been increased in calculating the results as percentage of dry matter (Table II), but, generally speaking, the discrepancies are probably of minor importance in the analyses of such heterogeneous material as silage, because the sampling errors are necessarily considerable, and attention is directed to the relative rather than to the absolute amounts of the products of biochemical action.

*Preparation of aqueous extract and* pH *measurement.* Duplicate samples of 100 g. of the fresh material were shaken with 200 c.c. $CO_2$-free water in wide-necked bottles for 3–4 hr. The liquid was squeezed out through cheese cloth and filtered. A portion of the filtrate was taken for the determination of *p*H by means of a quinhydrone electrode. 60 c.c. of the remainder were made up to 200 c.c. with neutralized 90 % alcohol, and, after standing overnight, the precipitate was filtered off. The determinations of acids and soluble nitrogenous compounds were made on this alcoholic solution by Foreman's method (1920) as employed by Woodman (1925).

*Volatile bases, amino-acids and volatile acids.* The *total acidity* was first determined by titrating 10 c.c. of the alcoholic solution and 50 c.c. neutral alcohol, enough to make the solution about 85 % alcohol, with $N/10$ sodium hydroxide. The calculated amount of alkali was added to a 50 c.c. portion of the alcoholic solution, which was then distilled in steam for 6–7 min., the distillate of alcohol and volatile bases being led into $N/10$ sulphuric acid. The bases were determined by titrating the excess of acid with alkali, using alizarin as indicator.

The alkalinity of the residue in the distilling flask was neutralized with acid, using phenolphthalein as indicator, to give a measure of the amino-acids present. (This determination was sometimes difficult on

account of the colour of the silage extract, but it was found that the colour change might be observed more easily by carrying out the titrations of duplicate samples concurrently by small stages.) The addition of acid was continued until the total acid was equivalent to the alkali originally added, and the solution was again distilled in steam. The volatile acids in the distillate were determined by titration with $N/10$ sodium hydroxide in presence of phenolphthalein.

If the volume of liquid in the distilling flask is kept constant, the whole of any butyric acid which is present will be distilled in the first 400 c.c. and approximately 90% of the acetic acid in the first 600 c.c. Lactic acid, however, is partially volatile in steam, as shown by Allen & Harrison (1935) and Cunningham & Smith (1937), so that a single distillation is liable to give erroneous results. For precise information, therefore, the steam distillation should be continued until a constant low titration value is obtained for successive equal portions of the distillate; the whole of the neutralized distillate should then be concentrated to small bulk, acidified with the appropriate amount of sulphuric acid, and redistilled in steam. Such a procedure is very laborious and protracted for routine purposes, and it has been found in practice that about 600 c.c. of the first distillate is likely to give a fairly reliable estimate of the volatile acids in silage by a compensation of errors.

The percentage of moisture ($M$) present in the fresh silage was taken into consideration in calculating the results given in Table I; for example, the titration values were multiplied by $\frac{200 \times (200 + M)}{10 \times 60}$ and $\frac{200 \times (200 + M)}{50 \times 60}$ to give respectively the total acidity and other values as percentages of the fresh silage.

The figure for residual acidity was obtained by subtracting the values for the volatile and amino-acids from the total acidity value.

*Lactic acid.* Watson & Ferguson (1937) showed that residual acidity may be taken only as a rough guide to the amount of lactic acid, and the results in Table II indicate that the differences between the residual acidity and lactic acid, as determined by analysis, are frequently considerable and liable to alter completely the ratio non-volatile : volatile acid.

It was necessary to determine the lactic acid in the aqueous alcoholic extract of the silage, and the following procedure, based on the work of a number of investigators, was finally adopted. It amounted to the precipitation of interfering substances by the method employed by Troy

& Sharp (1935) in the analysis of milk, oxidation of the lactic acid by the mixture recommended by Friedemann & Kendall (1929), and determination of the acetaldehyde by the technique described by Parkinson & Wagner (1934).

To get rid of the alcohol, 50 or 100 c.c. of the solution were neutralized with a few drops of 20 % caustic soda, diluted somewhat, and evaporated to small bulk. A few drops of dilute sulphuric acid were added to the solution, which was then made up to about 100 c.c. in a 200 c.c. flask. To this was added 0·5 c.c. of 20 % copper sulphate solution, and the liquid was heated to 46 or 47° C. A further quantity of 9·5 c.c. copper sulphate was then added, and the liquid kept at about 47° C. for 10 min. before the addition of 10 c.c. of a 30 % suspension of calcium hydroxide. The mixture was made up to about 201 c.c. with warm water and kept at 47° C., with frequent shaking, for a further 10 min. On cooling to room temperature, the suspension could be filtered rapidly to give a clear solution with a negative reaction to the Molisch test. A definite volume of the filtrate was neutralized with sulphuric acid in a distilling flask, and solutions of manganous sulphate and phosphoric acid were added so that the final volume contained 1 % $MnSO_4$ and was 0·1 *M* with respect to $H_3PO_4$. The liquid was boiled for a few moments before commencing the addition of approximately *N*/300 potassium permanganate drop by drop from a funnel, and the distillate was collected in *N*/20 sodium bisulphite. Distillation was continued until the contents of the distilling flask were brownish yellow—usually 15–30 min. for 2–10 mg. lactic acid. A definite volume of *N*/40 iodine was added to the receiving flask, and the excess immediately titrated with *N*/100 sodium thiosulphate. A blank experiment was carried out each time, and the amount of lactic acid was equivalent to the difference between the two quantities of thiosulphate required. (1 c.c. *N*/100 thiosulphate ≡ 0·45 mg. lactic acid.) By this method, the recovery of 5–10 mg. lactic acid, from solutions of zinc lactate, was about 93 %; from zinc lactate added to silage extracts, about 86 %. It is probable that the rather bulky precipitate of copper hydroxide absorbs some of the lactic acid, but the recovery is quite satisfactory for a method which is rapid and gives excellent agreement among replicate determinations.

## Table I. *Nitrogen, dry matter and acidity of various silages*

| Group | No. | *p*H | % dry matter | % crude protein | c.c. *N*/10 per 100 g. fresh silage: Total acidity | Amino-acids | Volatile acids | Volatile bases |
|---|---|---|---|---|---|---|---|---|
| 1. Fresh grass mixture | 6 | — | 30·1 | 10·5 | 57 | 17 | 4·7 | 4·5 |
| 2. Fresh legume mixture | 2 | — | 16·7 | 21·9 | 100 | 14 | 7·5 | 6·3 |
| A.I.V.: | | | | | | | | |
| 3. Legumes, *p*H 2·9–3·6 | 10 | 3·3 | 16·1 | 19·3 | 227 | 32 | 31 | 17 |
| 4. Grass, *p*H 3·1–3·8 | 11 | 3·4 | 20·6 | 14·0 | 129 | 37 | 35 | 12 |
| 5. Grass, *p*H 3·6–4·5 | 12 | 3·9 | 18·0 | 13·0 | 195 | 46 | 36 | 21 |
| 6. Grass, *p*H 3·8–4·7 | 6 | 4·1 | 20·7 | 13·1 | 207 | 43 | 60 | 17 |
| Pit and trench: | | | | | | | | |
| 7. Grass, *p*H 4·1–4·6 | 12 | 4·4 | 29·5 | 10·2 | 233 | 57 | 70 | 27 |
| 8. Grass, *p*H 4·5–5·0 | 10 | 4·8 | 21·5 | 12·4 | 178 | 30 | 113 | 62 |
| Stack: | | | | | | | | |
| 9. Grass, *p*H 4·5–4·6 | 3 | 4·5 | 29·5 | 11·9 | 123 | 6·7 | 59 | 30 |
| 10. Grass, *p*H 4·8–5·2 | 4 | 5·0 | 25·5 | 13·0 | 173 | 30 | 109 | 63 |
| 11. Grass, *p*H 5·0–5·1 | 3 | 5·1 | 23·0 | 9·9 | 181 | 8·9 | 151 | 72 |
| 12*a*. Oats, legume, *p*H 4·4 | 2 | 4·4 | 23·8 | 12·1 | 179 | 20 | 69 | 30 |
| 12*b*. Oats, legume, *p*H 4·3 | 1 | 4·3 | 28·2 | 10·3 | 323 | 82 | 130 | 57 |
| Tower: | | | | | | | | |
| 13. Oats, tare, *p*H 3·7–3·8 | 2 | 3·8 | 25·8 | 13·9 | 484 | 85 | 90 | 41 |
| 14. Oats, tare, *p*H 5·0 | 1 | 5·0 | 20·4 | 13·4 | 338 | 114 | 193 | 75 |
| Molasses: | | | | | | | | |
| 15. Grass, *p*H 3·7–4·6 | 3 | 4·0 | 26·5 | 10·8 | 305 | 30 | 93 | 19 |
| 16. Grass, *p*H 5·0–5·7 | 3 | 5·2 | 17·5 | 11·2 | 117 | 12 | 89 | 40 |
| 17. Grass, *p*H 6·9–7·4 | 4 | 7·2 | 16·5 | 9·7 | 19 | 2·6 | 1·6 | 1·1 |
| 18. Pit-grass, *p*H 5·6 | 1 | 5·6 | 21·8 | 13·1 | 118 | 30 | 43 | 46 |
| 19. Pit-grass, *p*H 6·0 | 1 | 6·0 | 33·2 | 19·2 | 80 | 5·3 | 4·6 | 36 |
| 20. Pit-grass, *p*H 6·4 | 1 | 6·4 | 18·9 | 13·9 | 90 | 7·7 | 67·3 | 59 |
| 21. Pit-grass, *p*H 7·9 | 1 | 7·9 | 19·5 | 14·8 | 11 | 6·0 | 4·7 | 3·0 |

## Table II. *Organic acids and distribution of nitrogen in silages*

| Group | % acids in fresh silage: Volatile as acetic | Residual as lactic | Lactic determined | Ratio Lactic/Acetic | % nitrogen in dry matter: Total nitrogen | Amino-acids | Volatile bases | Ratio Amino-acids/Volatile bases |
|---|---|---|---|---|---|---|---|---|
| 1 | 0·028 | 0·31 | 0·17 | 6·1 | 1·68 | 0·080 | 0·021 | 3·8 |
| 2 | 0·045 | 0·70 | 0·14 | 3·1 | 3·50 | 0·11 | 0·053 | 2·1 |
| 3 | 0·18 | 1·48 | 0·74 | 4·1 | 3·09 | 0·28 | 0·14 | 2·0 |
| 4 | 0·21 | 1·08 | 0·79 | 3·8 | 2·23 | 0·25 | 0·082 | 3·1 |
| 5 | 0·22 | 1·02 | 0·92 | 4·2 | 2·08 | 0·36 | 0·16 | 2·3 |
| 6 | 0·36 | 0·93 | 0·56 | 1·6 | 2·02 | 0·29 | 0·12 | 2·5 |
| 7 | 0·42 | 0·96 | 0·88 | 2·1 | 1·63 | 0·26 | 0·13 | 2·0 |
| 8 | 0·69 | 0·31 | 0·21 | 0·29 | 1·98 | 0·20 | 0·40 | 0·50 |
| 9 | 0·35 | 0·52 | 0·27 | 0·77 | 1·90 | 0·032 | 0·14 | 0·23 |
| 10 | 0·65 | 0·32 | 0·24 | 0·37 | 2·08 | 0·16 | 0·35 | 0·46 |
| 11 | 0·91 | 0·19 | 0·018 | 0·02 | 1·58 | 0·052 | 0·44 | 0·12 |
| 12*a* | 0·41 | 0·82 | 0·39 | 0·95 | 1·94 | 0·12 | 0·17 | 0·71 |
| 12*b* | 0·78 | 0·99 | 1·20 | 1·5 | 1·65 | 0·41 | 0·28 | 1·5 |
| 13 | 0·54 | 2·78 | 3·49 | 6·4 | 2·22 | 0·45 | 0·22 | 2·1 |
| 14 | 1·16 | 0·29 | 0·08 | 0·07 | 2·14 | 0·78 | 0·51 | 1·5 |
| 15 | 0·56 | 1·64 | 1·77 | 3·5 | 1·73 | 0·16 | 0·098 | 1·6 |
| 16 | 0·53 | 0·15 | 0·11 | 0·21 | 1·79 | 0·091 | 0·32 | 0·28 |
| 17 | 0·01 | 0·13 | 0·04 | 4·2 | 1·55 | 0·022 | 0·009 | 2·4 |
| 18 | 0·26 | 0·41 | 0·22 | 0·85 | 2·10 | 0·19 | 0·29 | 0·66 |
| 19 | 0·028 | 0·63 | 0·017 | 0·61 | 3·07 | 0·022 | 0·15 | 0·15 |
| 20 | 0·40 | 0·14 | 0·041 | 0·10 | 2·22 | 0·058 | 0·44 | 0·13 |
| 21 | 0·028 | 0·003 | 0·013 | 0·46 | 2·37 | 0·043 | 0·022 | 2·0 |

## DISCUSSION OF RESULTS

The *p*H values of the A.I.V. samples lie between 2·9 and 4·7 but, for the majority of the samples, the value is about 3·6 or 3·7 and, therefore, in accordance with the degree of acidity desired in this process. With the exception of a few odd samples, which are included at the foot of Tables I and II, and which will be considered separately, the other silages have values ranging from 3·7 to 5·7, but about 90 % are under *p*H 5·0. Possibly, as a result of the rather narrow range in *p*H, there is no obvious relationship between *p*H and total acidity in the case of the A.I.V. samples, and only a general tendency for a decrease in total acidity with a rise in *p*H value in the case of the other samples. In most groups, the figures for total acidity are much smaller than those obtained by Watson & Ferguson (1937), which may be due to the fact that they employed a greater proportion of water in preparing the silage extracts.

The percentage of dry matter lies between 16 and 21 for the A.I.V. samples and 16–30 for the others, whilst, for all samples, the percentage of crude protein varied from 10 to 20. The results for amino-acids and volatile bases have been expressed in terms of percentage nitrogen in the dry matter in order the better to observe the nature and extent of the protein breakdown. Bearing in mind that the figures have not been corrected for loss of volatile constituents during drying, it may be seen that, in groups 3–6, the amino-acids account for about 9–18 % of the crude protein and that the ratio of amino-acids to volatile base is between 2 and 3. This may be regarded as very satisfactory, especially in the case of group 3 with 3·1 % nitrogen. These results are in general agreement with the data for A.I.V. silage quoted by Peterson *et al.* (1935). Other groups in which the ratio is over unity are 7, 12*b*, 13 and 15 where the *p*H values lie between 3·8 and 4·4, and 14, a sample of oats and tares from a tower silo, with a *p*H of 5·0. The other six groups, 8, 9, 10, 11, 12*a* and 16, all contain more volatile base than amino-acid, the most notable in this respect being group 11 of stack silage, with volatile base accounting for more than 25 % of the total nitrogen. It appears, however, that the low ratio in these groups is generally due to a low content of amino-acid rather than to a high percentage of volatile base. The numbers of samples in the different groups are too small to justify any strict comparison between the *p*H values and the volatile base contents, but it is obvious that the critical zone occurs about *p*H 4·5.

With respect to the formation of organic acids in the different silages, the increase in volatile acids with the *p*H value in each group is clearly

shown. Butyric acid was not determined in any of the samples, although its presence was sometimes obvious in some of the samples of inferior quality. Figures for the residual acidity, calculated as lactic acid, are placed alongside those for lactic acid as determined directly by analysis, and the discrepancy is marked. A certain proportion of the residual acidity is, of course, due to mineral acids in the case of the A.I.V. samples, and the lower the $p$H value the greater this proportion might be expected to be. This is borne out by the results for groups 3, 4 and 5; the figures averaged for group 6 were not very uniform and less reliance can be placed on them. The percentage of lactic acid in the other silages varies considerably, but in each group the ratio lactic to acetic acid decreases as the $p$H value rises. There is a striking resemblance between these results and the figures obtained by Watson & Ferguson. They used the figure for residual acidity in their calculations, but, as mentioned above, they found larger percentages of volatile acids, so that the agreement may be more apparent than real. It will be observed that, with one exception, the lactic acid is greater than volatile acid in the same groups as contain more amino-acid than volatile base, a $p$H value of about 4·5 again being the critical point. The exception is the single sample of 1931 tower silage (group 14), which had lain under subsequent additions to the tower for 3 years, and contains a large amount of volatile acids and very little lactic acid.

The figures given for the fresh material (groups 1 and 2) are of interest inasmuch as they give some guide to the composition of the crops ensiled as revealed by the analytical methods used in the examination of the silages. The amounts of material extracted are, of course, very small and are liable to considerable error, but they indicate that ensilage has increased the amounts of organic acids and non-protein nitrogen with only one or two unimportant exceptions.

The odd samples, 17–21, for example, with $p$H values of 5·6–7·9 for pit silage and 7·2 for molasses silage, give results which are irregular. Samples 18, 19 and 20 show the usual decrease in lactic : acetic acid and amino-acid : volatile base ratios with increase in $p$H. In fact, 18 is not unlike 8 or 10, and 20 is similar to 11 in respect to the absolute amounts of the decomposition products, but 17 and 21 display unusual features. As has been pointed out by Woodman & Amos (1924), it is probable that secondary changes have taken place, and the ratios in 17 and 21 are misleading. Besides having a characteristic musty smell, the silages of high $p$H were so dispersed as to make it extremely difficult to prepare an aqueous extract.

SUMMARY

The chemical composition of eight samples of fresh material and ninety-one samples of different kinds of silage has been determined. The crude protein varied from about 10 to 20 %. Judging by the appearance and edibility of the material, the silage samples made by the A.I.V. process were almost uniformly good. Their *p*H values were mainly between 3·5 and 3·8, and they contained less volatile acids and volatile bases than the other silages, and showed the highest proportions of lactic to acetic acid and amino-acids to volatile bases. Two exceptions to this were found in some samples of oats-tare tower silage and grass-molasses silage, with *p*H values about 4, which contained large amounts of lactic acid and small amounts of volatile bases, and, on the basis of the above-mentioned ratios, were as good as the A.I.V. silage. Nearly half of the samples of pit and stack silages had *p*H values below 4·5, and also contained more lactic than acetic acid, and more amino-acids than volatile bases. The results as a whole show a fairly close agreement between *p*H, volatile acids and volatile bases, but attention is directed to the possibility of anomalous figures when, through secondary changes, the *p*H value has risen to about 6 or 7.

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# STUDIES IN SOIL CULTIVATION

## VII. THE EFFECT OF CULTIVATION ON CROP YIELD


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### Introduction

Cultivation methods were developed in the days when horses or oxen were the only sources of motive power. Except at the busiest times of the year, some men and horses would be free, and when circumstances permitted it was natural to use them on the land in the belief that if an operation improved the appearance of the soil the crops must benefit. Hence there may be two kinds of cultivation operations, those essential for good crops and those that are really spare-time occupations. Many of the latter have become merged in the body of agricultural tradition, so that it is not now possible without experiment to distinguish between essential and subsidiary operations. Two important features of modern arable farming are the rapid rise in the cost of agricultural labour compared with the value of the agricultural products sold or consumed on the farm, and the partial replacement of the horse by the tractor. To enable the wage scales to be paid the output per man must be increased and the cost of cultivation reduced as much as possible. One of the main differences between the horse and the tractor is that the horse costs money every day of the year while the tractor, if properly looked after, need cost money only when it is running. Production costs are now a much more fundamental aspect of British agriculture than before, so it is often profitable to cut out some operations even if the yields are slightly reduced. Hence one urgent problem in modern mechanized farming is to discover what, if any, loss of yield occurs when certain cultivation operations are omitted, or when cheaper and more rapid methods are substituted for time-honoured but more expensive ones.

Investigations on the effects of cultivation on crop yield thus fall naturally into two categories: those that are accepted as necessary, such as preparing a seed bed, and those that are considered desirable but are not absolutely essential. In each category one must consider the effects

of altering the methods of cultivation for one year only and the cumulative effects if they are altered over a period of years.

Experiments on these subjects have been carried out on the Rothamsted Experimental Farm since 1926, and sufficient data have now accumulated to make a summary of the results worth while. The main conclusions reached are that in any one year the farmer can, with little if any loss of crop, cut out or telescope a number of cultivation operations, but if he tries to prepare a seed bed for several years in succession by more rapid methods than by beginning with the traditional ploughing he may run into certain secondary troubles.

## I. THE INFLUENCE OF THE SEED BED ON CROP YIELD

Experiments have been in progress on the experimental farm on the comparison of seed beds prepared by using the plough and harrows and by using a Rototiller.[1] The results obtained for the years 1926–9 have already been discussed by Keen *et al.* (1930). The comparison during these years was only for the spring-sown crops of swedes and barley. In every case the land was winter ploughed first and in the experiments the plots were either reploughed or worked with the Rototiller on the stale furrow. The results were that in each experiment the ploughed plots yielded slightly better than those rototilled, though the differences were usually small. From 1930 onwards the Rototiller was used on the stubble direct and in 1932 the experiments were extended to compare these two implements working at two different depths, and in 1933 again extended to include the cultivator or grubber[2] as a third implement for preparing a seed bed.

### (A) *Description of the experiments carried out from 1930 to 1936 at Rothamsted*

*Year* 1930. Mangolds, previous crop swedes. A comparison was made between ploughing, rototilling, and rototilling with a ridging body attached behind the machine. All the plots received their first cultivation

[1] A Rototiller supplied by Messrs Geo. Munro Ltd. of Waltham Cross was used in these experiments.

[2] In this and the following paper we have deliberately used the less common name "grubber" instead of the commoner name "cultivator". The word cultivator has no verb available corresponding to the verbs to plough, to harrow and to roll, for to cultivate means to till and not to work with a cultivator. But by using the name grubber we can use the verb to grub. The tractor cultivator used was, in fact, a grubber according to the definition given by J. R. Bond in his book *Farm Implements and Machinery* (Benn Bros. 1923).

in February, and were recultivated in the same way a second time in the beginning of May after they had all been rolled and harrowed. Subsequently the ploughed plots were harrowed and the whole was drilled with Yellow Globe mangolds.

*Year* 1930–1. Winter wheat, previous crop winter oats. A comparison was made between ploughing with an ordinary plough, ploughing with the Massey Harris pulverator plough,[1] each being followed by sufficient subsequent cultivations to obtain a good seed bed, and using a Rototiller alone. The land was worked in rather bad condition and some of the ordinary ploughed plots had to be followed by as many as six subsequent cultivations to obtain a good enough seed bed; the pulverator needed from two to four and the Rototiller none. On this experiment was superimposed one to determine the effect of the spring cultivations of harrowing and rolling on crop yield, which will be discussed in § III (A) p. 227.

*Year* 1932–3. Winter wheat (Pastures Field). The design and description of this experiment have been given by Keen (1933). A comparison was made between the plough and harrows and the Rototiller each working either at 4 or 8 in. depth on each of which was superimposed the effect of a top dressing of sulphate of ammonia and of the spring cultivations of rolling and harrowing. The Rototiller had to go over a plot twice to work down to 8 in. owing to the low power of the engine and the heaviness of the soil. The experimental results of the spring cultivation experiment will be discussed in § III (A) (p. 227).

*Year* 1933–4. Winter wheat (Pastures Field). The 1932–3 experiment was continued for a second year; but the effects of spring cultivation were not included. Instead, half the plots ploughed in 1932 were ploughed in 1933 and the other half rototilled, and similarly half the plots rototilled in 1932 were rototilled in 1933 and the other half ploughed.

*Year* 1933 *onwards*. Long Hoos rotation experiment. This is a new long-term rotation experiment. It consists of three sections in which wheat grows in one, mangolds in another, and barley in the third, and the rotation of crops in each section is in the order given. Each section is divided into four blocks. In each block there are twelve treatments, namely ploughed and harrowed, grubbed and harrowed, and rototilled, all working either to 4 or 8 in. depth and receiving either calcium cyanamide

[1] The pulverator plough has a very short breast behind which is a rotating vertical shaft fitted with five blades. These blades shatter the furrow slice as it is turning over, leaving a much more broken surface. The plough was kindly lent by Messrs Massey Harris, Ltd.

or nitrochalk as the nitrogenous fertilizer. The plots in two of these blocks, called the "Continuous series", receive the same treatment every year, but in the other two blocks, called the "Rotating series", the treatments follow a regular rotation. On one of the rotating blocks the cultivation rotation, called rotation A, is ploughing one year, rototilling the next and grubbing the third, while in the other block rotation B is followed, namely ploughing one year, grubbing the next, and rototilling the third. In these two blocks the depth of cultivation is changed every year so that those plots cultivated deep one year are cultivated shallow the next, while the fertilizer is changed every second year so that half the plots receive cyanamide for two years in succession and then nitrochalk for the next two years while the other half receive nitrochalk for these two years and cyanamide for the next two years.

For the wheat break the barley stubble receives the appropriate cultivation in the late autumn. The ploughed and grubbed plots are harrowed before drilling and the whole field is harrowed after drilling. For barley after mangolds the plots to be ploughed or grubbed are so treated if practicable after the mangolds have been carted off, and in the spring all the plots receive their appropriate cultivations, so that in general the ploughed and the grubbed series are worked twice while the rototilled series is worked only once. The ploughed and grubbed plots are harrowed and rolled as necessary. For mangolds after wheat neither the grubber nor the Rototiller was able to bury the weeds very well; during the first two years' experiments the weeds almost choked the young mangolds on these plots, and it was decided that from 1935 onwards the wheat stubble should be shallow ploughed early in the autumn to cover as many of the last season's weeds as possible.

The lay-out of the experiment consists of two parallel rows of 72 plots each, separated by a strip 40 links (8·05 m.) wide used as a headland. The plots are 139·8 links long by 11 links wide (28·12 by 2·21 m.), thus having an area of 1/65 acre (62·2 sq. m.) per plot. There are 2-link (0·4 m.) paths between the plots in each crop and 5-link (1·0 m.) paths between crops.

*Limitations in the present experimental series.* Two limitations have arisen connected respectively with the lay-out and the time of cultivation. Long narrow plots give, as is now well known, more accurate results in complex experiments than square plots. They are also suited to cultivating land in one direction, as it is possible to have large headlands. But it is absolutely impossible to cross-cultivate selected plots. Either all the plots in a block can be cross-cultivated or none can be.

The second limitation is that so far the main seed-bed cultivations have been done on the same day. This is very convenient from the point of view of farm management, for work on the comparative differences in soil structure produced by the different implements, and for ensuring that the sowing date is the same for the whole experiment. But it has sometimes involved working the land when it has been in a condition not best suited to one or two of the implements: the ideal condition of the soil for these three methods of cultivation to produce their best results obviously need not be the same. This criticism is not necessarily important, for the farmer has not always the time, the staff, or the implements necessary to cultivate his land only when it is in what he considers an ideal condition. He must often use his implements under other conditions just because he wants to get a crop in by a certain time. He will often choose to take unsuitable existing conditions rather than wait in the hope that better will follow.

### (B) *The Experimental Results*

The yields of the individual plots and the dates the various agricultural operations were carried out are all given in the appropriate Rothamsted Reports. In this section, for the sake of brevity, only mean yields or differences between mean yields will in general be given.

*Preliminary experiments comparing the plough and Rototiller.* Up to 1929 the implements only worked on a stale furrow. In 1930 and 1931 the Rototiller worked on the stubble direct. The results of these experiments are given in Table I.

Table I. *Comparison between the yields of crops grown on seed bed prepared either by the plough or by the Rototiller*

| | | Decrease due to Rototiller compared with plough and harrows | | |
|---|---|---|---|---|
| Year | Crop | Mean | S.E. of mean | Mean yield |
| 1926 | Swedes, roots (tons/acre) | 1·65 | 0·57 | 10·22 |
| 1928 | Swedes, roots (tons/acre) | 2·55 | 0·71 | 21·40 |
| 1929 | Barley, grain (cwt./acre) | 0·6 | 0·81 | 30·2 |
| | Barley, straw (cwt./acre) | 1·2 | 1·68 | 44·3 |
| 1930 | Mangolds, roots (tons/acre) | 3·78 | 2·94 | 28·97 |
| 1931 | Wheat, grain (cwt./acre) | 1·5 | 0·73 | 15·8 |
| | Wheat, straw (cwt./acre) | 1·9 | 2·69 | 39·1 |

In each experiment the yield on the rototilled plots was less than on the ploughed plots, but the reduction was only significant in the two swede experiments.

*Wheat.* The yields of winter wheat in the 1932–3 and 1933–4 experiments on Pastures Field are given in Table II.

Table II. *Yield of winter wheat on Pastures Field*

| Year | | Ploughed Deep | Ploughed Shallow | Rototilled Deep | Rototilled Shallow | S.E. per treatment |
|---|---|---|---|---|---|---|
| 1932–3 | Grain (cwt./acre) | 24·0 | 23·3 | 23·6 | 22·0 | 0·78 |
| | Straw (cwt./acre) | 35·9 | 33·7 | 33·2 | 33·1 | 1·48 |
| 1933–4 | Grain (cwt./acre) | 12·1 | 10·6 | 12·2 | 8·6 | 0·85 |
| | Straw (cwt./acre) | 15·5 | 13·6 | 14·5 | 10·3 | — |

In both years there was no real difference in yield between the deep-rototilled and the deep-ploughed plots. In 1932–3 the shallow-rototilled plots yielded just a little less than the others, but in 1933–4 they yielded very much less than the deep-tilled plots.

The effect of the tillage carried out in the autumn of 1932 on the wheat yield in the harvest year 1934 is negligible as is shown in Table III, which gives the yield of grain in 1934 in cwt./acre.

Table III. *The effect of tillage for the previous crop on the yield of wheat in the following crop* (*Pastures Field*)

Yield of wheat grain in cwt./acre.

| | Ploughed, autumn 1933 Deep | Ploughed, autumn 1933 Shallow | Rototilled, autumn 1933 Deep | Rototilled, autumn 1933 Shallow |
|---|---|---|---|---|
| Ploughed, autumn 1932 | 12·2 | 10·6 | 12·6 | 8·5 |
| Rototilled, autumn 1932 | 12·0 | 10·6 | 11·7 | 8·6 |
| Plough – rototiller, 1932 | 0·2 | 0·0 | 0·9 | −0·1 |

Standard error of each difference = 1·7 cwt./acre.

The effect of the top dressing of sulphate of ammonia was negligible on the grain yield in each year for each cultivation treatment and on the straw yield in 1933–4. But it increased the yield of straw on each treatment by about 4 cwt. an acre in 1932–3.

The 1934 experiment gave a very disappointing yield, averaging only just under 11 cwt./acre. This experiment will be discussed more fully in the following paper when the growth of the crop is followed in detail. It will be shown there that the number of wheat plants is about half that on a normal stand of wheat and the slight increase of tillering made little difference to the number of shoots bearing earheads at harvest. The plants were individually weak as the yield per plant was below average.

The yields of wheat on the Long Hoos cultivation xperiments for the three seasons 1933–6 are given in Table IV.

Table IV. *Yield of wheat on Long Hoos rotation cultivation experiment*

| Cultivation treatment | | Continuous series Plough | Continuous series Rototiller | Continuous series Grubber | Rotating series Plough | Rotating series Rototiller | Rotating series Grubber |
|---|---|---|---|---|---|---|---|
| Grain (cwt./acre): | | | | | | | |
| 1933–4* | Deep | — | — | — | 25·1 | 22·5 | 25·1 |
| | Shallow | — | — | — | 24·9 | 19·8 | 23·0 |
| | | | | Standard error per treatment = 0·71. | | | |
| 1934–5 | Deep | 22·1 | 19·5 | 20·6 | 23·0 | 22·2 | 19·5 |
| | Shallow | 24·7 | 21·6 | 19·6 | 21·9 | 20·0 | 16·8 |
| | | Standard error per treatment = 1·17. | | | | | |
| 1935–6† | Deep | 22·3 | 21·2 | 21·2 | 20·9 | 20·6 | 20·0 |
| | Shallow | 22·8 | 21·3 | 20·5 | 20·8 | 20·5 | 19·8 |
| | | Standard error per treatment = 0·68. | | | | | |
| Straw (cwt./acre): | | | | | | | |
| 1933–4* | Deep | — | — | — | 30·3 | 27·1 | 29·7 |
| | Shallow | — | — | — | 29·4 | 24·6 | 27·1 |
| | | | | Standard error per treatment = 0·75. | | | |
| 1934–5 | Deep | 34·4 | 33·0 | 33·8 | 34·7 | 36·0 | 33·2 |
| | Shallow | 39·2 | 34·8 | 32·1 | 35·0 | 32·0 | 29·3 |
| | | Standard error per treatment = 2·68. | | | | | |
| 1935–6† | Deep | 49·4 | 43·7 | 42·3 | 41·3 | 41·0 | 36·4 |
| | Shallow | 46·6 | 42·5 | 38·8 | 44·0 | 42·9 | 40·5 |
| | | Standard error per treatment = 1·98. | | | | | |

* 1933–4 was the first year of the experiment so that there was no difference in the cultivation treatments of the continuous and the rotating series. This footnote applies also to the corresponding entries in Tables VII and X.

† 1935–6. The winter wheat had failed so a spring-tine harrow was put across the field on 17 March 1936 and it was re-drilled on 18 March with Little Joss wheat.

The results obtained on Pastures Field and Long Hoos are summarized in Tables V and VI.

Table V. *Beneficial effect of deep compared with shallow tillage for wheat*

| | Plough | Rototiller | Grubber |
|---|---|---|---|
| Grain (cwt./acre) | | | |
| Group 1. After commercial farming | 0·5 | 2·2 | 2·1 |
| Group 2. Rotating series | 0·8 | 2·1 | 1·5 |
| Group 3. Continuous series | −0·3 | 0·3 | 0·8 |
| **Mean** | 0·3 | 1·5 | 1·4 |
| Straw (cwt./acre) | | | |
| Group 1. After commercial farming | 1·5 | 1·3 | 2·6 |
| Group 2. Rotating series | −1·0 | 2·3 | 0·0 |
| Group 3. Continuous series | 0·2 | 1·0 | 2·6 |
| **Mean** | 0·2 | 1·5 | 1·7 |

Table VI. *Beneficial effect of plough (P) compared with Rototiller (R) and grubber (G)*

| | Deep | | Shallow | |
|---|---|---|---|---|
| | P–R | P–G | P–R | P–G |
| Grain (cwt./acre) | | | | |
| Group 1. After commercial farming | 1·6 | 0·0 | 3·2 | 1·9 |
| Group 2. Rotating series | 0·2 | 2·1 | 1·4 | 3·1 |
| Group 3. Continuous series | 1·4 | 1·3 | 2·0 | 3·4 |
| Mean | 1·1 | 1·1 | 2·2 | 2·8 |
| Straw (cwt./acre) | | | | |
| Group 1. After commercial farming | 2·9 | 0·6 | 2·7 | 2·3 |
| Group 2. Rotating series | 0·0 | 3·1 | 2·6 | 4·6 |
| Group 3. Continuous series | 3·0 | 3·8 | 3·8 | 7·4 |
| Mean | 2·0 | 2·5 | 3·0 | 4·8 |

The result of these experiments is again that ploughing gives fairly consistently about 1–2 cwt. more grain and 2–4 cwt. more straw per acre than does rototilling or grubbing. There is no virtue in ploughing deeper than 4 in., but it is advantageous to use both the Rototiller and grubber to 8 in. if possible. There is, so far, no sign that yields of grain are seriously affected when the same cultivation implement is used on the land for three years running, for the yields on the continuously grubbed or rototilled plots in 1936 compared with the plough were no worse than

Table VII. *Yield of barley on the Long Hoos cultivation experiment*

| Cultivation treatment | | Continuous series | | | Rotating series | | |
|---|---|---|---|---|---|---|---|
| | | Plough | Rototiller | Grubber | Plough | Rototiller | Grubber |
| Grain (cwt./acre): | | | | | | | |
| 1934 | Deep | — | — | — | 26·2 | 27·8 | 26·8 |
| | Shallow | — | — | — | 26·7 | 24·6 | 26·1 |
| | | | | | Standard error per treatment = 0·54. | | |
| 1935 | Deep | 35·6 | 36·0 | 35·4 | 34·5 | 34·8 | 35·0 |
| | Shallow | 37·2 | 35·9 | 33·8 | 33·5 | 33·3 | 33·6 |
| | | Standard error per treatment = 1·72. | | | | | |
| 1936 | Deep | 29·2 | 26·5 | 23·3 | 29·3 | 29·4 | 27·4 |
| | Shallow | 26·3 | 24·7 | 19·5 | 28·7 | 27·0 | 26·1 |
| | | Standard error per treatment = 0·96. | | | | | |
| Straw (cwt./acre): | | | | | | | |
| 1934 | Deep | — | — | — | 25·9 | 26·4 | 25·8 |
| | Shallow | — | — | — | 26·4 | 23·4 | 25·7 |
| | | | | | Standard error per treatment = 0·50. | | |
| 1935 | Deep | 42·0 | 40·0 | 38·7 | 39·8 | 38·0 | 39·7 |
| | Shallow | 41·3 | 40·2 | 37·4 | 38·4 | 36·6 | 37·7 |
| | | Standard error per treatment = 2·22. | | | | | |
| 1936 | Deep | 42·2 | 40·1 | 35·7 | 41·0 | 39·9 | 40·3 |
| | Shallow | 38·9 | 38·5 | 32·4 | 39·2 | 38·6 | 41·1 |
| | | Standard error per treatment = 1·21. | | | | | |

in 1935 or 1934. The yield of straw on the continuous shallow grubbed plots does, however, seem to be suffering.

*Barley.* The yields on the Long Hoos cultivation experiment for the years 1934–6 are given in Table VII and are summarized in Tables VIII and IX.

Table VIII. *Beneficial effect of deep compared with shallow tillage*

| | Plough | Rototiller | Grubber |
|---|---|---|---|
| Grain (cwt./acre): | | | |
| After commercial farming | −0·5 | 3·2 | 0·8 |
| Rotating series | 0·8 | 2·0 | 1·4 |
| Continuous series | 0·6 | 0·9 | 2·7 |
| Mean | 0·3 | 2·0 | 1·6 |
| Straw (cwt./acre): | | | |
| After commercial farming | −0·5 | 3·0 | 0·2 |
| Rotating series | 1·6 | 1·4 | 0·6 |
| Continuous series | 2·0 | 0·7 | 2·3 |
| Mean | 1·0 | 1·7 | 1·0 |

Table IX. *Beneficial effect of ploughing (P) compared with Rototiller (R) or grubber (G)*

| | Deep | | Shallow | |
|---|---|---|---|---|
| | P–R | P–G | P–R | P–G |
| Grain (cwt./acre): | | | | |
| After commercial farming | −1·6 | −0·6 | 2·1 | 0·6 |
| Rotating series | −0·2 | 0·7 | 1·0 | 1·3 |
| Continuous series | 1·1 | 3·0 | 1·3 | 5·1 |
| Mean | −0·2 | 1·0 | 1·5 | 2·3 |
| Straw (cwt./acre): | | | | |
| After commercial farming | −0·6 | 0·1 | 3·0 | 0·7 |
| Rotating series | 1·4 | 0·4 | 1·2 | −0·5 |
| Continuous series | 2·0 | 4·9 | 0·7 | 5·2 |
| Mean | 0·9 | 1·8 | 1·6 | 1·8 |

Here again it is obvious there is no virtue in using the plough deep though there is a distinct advantage in using the Rototiller and grubber deep. If the Rototiller is used deep it probably gives as good a yield as the plough, while the same result only holds true for the grubber if it is not used on the same land three years in succession. With shallow grubbing for two or three years in succession the yields are far below the rest. This progressive deterioration has not yet begun to show on the shallow rototilled plots.

*Mangolds.* In the Long Hoos experiment there are four rows of mangold plants per plot, with a spacing of 22 in. between the rows inside the plot but a spacing of 38 in. between the outside row of one plot and the outside row of the adjacent plot. In the following paper it will be

shown that the yields of the two outside rows are heavier than the inside rows because of the increased spacing on one side of each outside row. For this reason, in the discussion given here, only the yields of the inner rows have been used, the yields of the two outside edge rows being rejected. The yields of mangolds, calculated on this basis, are given in Table X and summarized in Tables XI and XII.

Table X. *Yield of mangolds on Long Hoos cultivation experiment (edge rows rejected)*

Roots (tons/acre)

| | Continuous series | | | Rotating series | | |
|---|---|---|---|---|---|---|
| | Plough | Rototiller | Grubber | Plough | Rototiller | Grubber |
| 1934 | — | — | — | 37·5 | 33·9 | 37·2 |
| | — | — | — | 36·5 | 34·2 | 36·2 |
| | | | | Standard error per treatment = 1·11. | | |
| 1935 | 25·0 | 22·8 | 21·8 | 20·6 | 21·0 | 19·5 |
| | 23·3 | 17·7 | 13·0 | 21·4 | 19·3 | 19·4 |
| | Standard error per treatment = 1·50. | | | | | |
| 1936 | 23·2 | 21·2 | 17·6 | 23·0 | 21·7 | 21·0 |
| | 21·7 | 18·3 | 18·3 | 22·1 | 19·2 | 20·4 |
| | Standard error per treatment = 0·94. | | | | | |

Table XI. *Beneficial effect of deep compared with shallow tillage*

Roots (tons/acre)

| | Plough | Rototiller | Grubber |
|---|---|---|---|
| After commercial farming | 1·0 | −0·3 | 1·0 |
| Rotating series | 0·0 | 2·0 | 0·4 |
| Continuous series | 1·7 | 4·0 | 4·0 |
| Mean | 0·9 | 1·9 | 1·8 |

Table XII. *Beneficial effect of ploughing (P) compared with the Rototiller (R) and grubber (G)*

Roots (tons/acre)

| | Deep | | Shallow | |
|---|---|---|---|---|
| | P–R | P–G | P–R | P–G |
| After commercial farming | 3·6 | 0·2 | 2·3 | 0·3 |
| Rotating series | 0·5 | 1·5 | 2·5 | 1·9 |
| Continuous series | 2·1 | 4·4 | 4·5 | 6·8 |
| Mean | 2·1 | 2·0 | 3·1 | 3·0 |

Again there appears to be no great virtue in ploughing deep, though it possibly increases the yield of roots by about 1 ton/acre. Nor does

there seem to be any great virtue in using the grubber or Rototiller deep if the land has been ploughed deep the previous year. It appears that if the Rototiller is used two or more years in succession it should be used deep, but the result for the grubber is not so consistent. On the continuous series in 1935 the shallow plots yielded 8·7 tons less and in 1936 0·7 tons more than the deep plots. The reduction of 8·7 tons was statistically significant. Thus it appears that deep cultivation with the grubber or Rototiller is never harmful, is usually beneficial, and may be very beneficial.

The plough usually gives the highest yield of mangolds. On land ploughed the previous year the decrease of yield using the grubber or Rototiller is of the order of 1–2 tons/acre; but when they are used two or more years in succession, the yields begin to fall off. The reductions in yield due to using the grubber instead of the plough were 3·2 and 5·6 tons/acre in 1935 and 1936 when used deep, and 10·2 and 3·3 tons/acre when used shallow, while the reductions for the Rototiller were 2·2 and 2·1 tons/acre when used deep and 5·6 and 3·3 tons/acre when used shallow. Clearly the grubbed plots are becoming less suited to mangolds than the ploughed plots, though there is little sign of progressive deterioration on the rototilled plots. The reason for the surprisingly low yields of the shallow grubbed plots on the continuous series in 1935 is not clear, for if it were due to the soil being in the wrong condition at the time of cultivation one would have expected the shallow grubbed plots in the rotating series to give an abnormally low yield. The reductions of yield on the rotating series in 1935 and 1936 due to using the grubber instead of the plough were 1·1 and 2·0 tons/acre if both were used deep and 2·0 and 1·7 tons/acre if both were used shallow, thus giving no abnormal depression for 1935.

*The pulverator plough.* An experiment was made in 1930–1 comparing the yields of winter wheat grown on seed beds prepared by the plough, the pulverator plough and the Rototiller. The results of this experiment are given in Table XIII.

Table XIII. *Comparison of the plough, the pulverator plough and the Rototiller*

| | Decrease in yield as compared with the pulverator plough and harrows when land is cultivated with | | | |
|---|---|---|---|---|
| | Plough | Rototiller | S.E. of difference | Mean yield |
| Grain (cwt./acre) | 1·1 | 2·6 | 0·73 | 15·8 |
| Straw (cwt./acre) | 0·7 | 2·6 | 2·69 | 39·1 |

The crop was rather badly lodged at harvest and some of the grain began sprouting while in the shocks. The increase of yield of the pulverator ploughed crop over the ordinary ploughed crop was not significant, though the decrease of yield on the rototilled plots compared with the pulverator ploughed plots was significant.

The only other comparisons of pulverator ploughed plots with ordinary ploughed plots known to the authors are those made by Davies (1931–2) on a loam soil on the Wye experimental farm. He found that on the whole the yield of winter- and spring-sown barley was highest on the ploughed, lowest on the rototilled, and intermediate on the pulverator ploughed plots. At neither of these two centres has there been any large effect due to the use of the pulverator.

*Conclusions.* The conclusions reached from these experiments are:

(1) It rarely appears necessary to plough deeper than 4 in.

(2) A seed bed prepared by a plough and harrows never gives a significantly lower yield than one prepared either by a Rototiller or a grubber and harrows, and often gives a better yield. But if time is important it is possible to dispense with ploughing for one year and to substitute a quicker method of preparing a seed bed with little if any loss of yield, particularly if the land is worked to 8 in. The reduction in yield will only rarely be more than 1 cwt./acre of grain or 2 tons/acre of mangolds.

(3) No harmful results of using the Rototiller year after year have yet come to light, provided it is worked to about 8 in. But the grubber does not appear to be a suitable implement to replace the plough for several years in succession, though if used to about 8 in. deep it may be satisfactory for several years with wheat or barley.

These conclusions are in harmony with those obtained by other workers elsewhere. Thus Sanders (1935) and Garner & Sanders (1936) at Cambridge, working on the light gravelly loam of the University Farm, compared the grubber with the plough for preparing a seed bed for winter wheat after potatoes in the four seasons 1929–33. In only one year did they obtain an appreciable difference of yield between the two treatments, and that was in 1930–1 when the ploughed plots gave 15 % more grain than the grubbed ones. Sanders (1935) has described the preliminary results obtained in a further series of experiments to compare the effect of working the land with a Fowler Gyrotiller with that obtained by using a plough. In 1934 working on this light gravelly loam the yields of sugar-beet on the gyrotilled and on the ploughed and subsoiled plots were practically identical. In 1933–4 and 1934–5, working on the heavy

clay land of the University Farm, they found no difference in the yields of winter wheat and winter beans on the gyrotilled and on the ploughed land.

These English results are in good agreement with the German ones. Tamm (1928) and Gade (1929) both found that rotary cultivation gave what they considered to be more desirable physical properties to the soil than the plough or grubber, yet to their surprise neither obtained any appreciable increase or decrease of yield. More recently von Nitzsch (1935, 1936) has made an analysis of the effect of different methods of cultivation on the physical properties of soils and on the crop yields. His results show that in general the farmer has a very wide latitude in the methods of preparing his seed bed if he chooses his time correctly, and that he can often save power and time by using a grubber or a rotary cultivator to prepare his seed bed instead of the plough with little effect on the crop yield.

## II. Effect of subsidiary cultivations of seed bed on yield

### (A) *The effect of subsoiling*

The object of these experiments was to ascertain if there is any advantage in loosening the subsoil when no obvious pan or plough sole is being formed. The Rothamsted soil is rather heavy but contains many flints and is remarkably permeable considering its high clay content.

In 1914 and 1916 two small experiments were carried out to find the effect of subsoiling on the yield of potatoes, and in each case subsoiling appeared to give an increase of about 11 cwt./acre. In these experiments the effects of soil heterogeneity were not eliminated and it appeared probable that one of the results at least was due to this cause. Starting in 1928 a new set of experiments was set up to test the effect of subsoiling on the yield of sugar-beet. In 1928 in a properly designed experiment the effect of ploughing and subsoiling to a depth of 14 in. was compared with simple ploughing. In 1931 small plots were used in which the subsoil was loosened by hand digging. The results of these two experiments are given in Table XIV.

Table XIV. *Effect of subsoiling on the yield of sugar-beet*

| | | Increase due to subsoiling | | |
|---|---|---|---|---|
| | | Mean | S.E. of mean | Mean yield |
| 1928 | Roots (tons/acre) | 0·03 | 0·14 | 9·15 |
| | Tops (tons/acre) | 0·40 | 0·30 | 11·43 |
| 1931 | Roots (tons/acre) | −0·23 | 0·15 | 12·66 |
| | Tops (tons/acre) | −0·09 | 0·29 | 15·95 |
| | Sugar (cwt./acre) | −0·4 | 0·57 | 48·6 |

There is thus no evidence that subsoiling or loosening the subsoil has any appreciable effect on either the yield of the sugar-beet or of sugar.

### (B) *The effect of an extra ploughing*

In the autumn of 1933 two experiments were laid out, one on potatoes to test the effect of ploughing in autumn and spring with ploughing in spring alone, and one on sugar-beet to test ploughing in autumn and spring with ploughing in autumn alone. The plots were long and narrow so the second ploughing could not be a cross-ploughing. The results for the potato experiment are given in Table XV.

Table XV. *The effect of twice ploughing the land compared with once ploughing it on the yield of potatoes* (1934)

| Spring manurial treatment | Increase in tons/acre due to autumn ploughing | | | | | Mean yield |
|---|---|---|---|---|---|---|
| Dung | None | Fresh | Rotted | Mean | S.E. of mean | |
| | 0·33 | 0·47 | −0·12 | 0·23 | 0·21 | 11·38 |
| Sulphate of ammonia | None | 0·4 cwt. N | 0·8 cwt. N | | | |
| | 0·00 | 0·19 | 0·05 | | | |

The autumn ploughing has clearly not increased the yield significantly in any treatment, although possibly it was somewhat more beneficial when a heavy dressing of sulphate of ammonia was given to the potatoes.

The sugar-beet experiment tested the effect of spring ploughing on land which had been ploughed the previous autumn. The experimental results are given in Table XVI.

Table XVI. *Effect of twice ploughing the land compared with once ploughing it on the yield of sugar-beet* (1934)

| Manurial treatment | Increase due to spring ploughing: No chloride | NaCl | KCl | NaCl + KCl | Mean | S.E. of mean | Mean yield |
|---|---|---|---|---|---|---|---|
| Roots (tons/acre) | 0·12 | 1·94 | 0·06 | −0·66 | 0·37 | 0·27 | 15·36 |
| Tops (tons/acre) | 0·24 | −0·08 | −0·21 | 1·56 | 0·38 | 0·31 | 14·36 |
| Sugar (cwt./acre) | 0·1 | 6·7 | 0·0 | −2·4 | 1·1 | 0·95 | 54·5 |

The only increase worth commenting on is that of the roots when sodium chloride alone was added. The increase is significant and appears to be reasonably consistent. It is formed from the mean of four separate additional treatments which gave increases of 1·43, 1·71, 4·20 and 0·44 tons/acre respectively, these increases being based on the means of two comparisons between spring and autumn ploughing with autumn ploughing alone. But it is a very odd result for it is not shown up either in the tops or in the roots in the presence of potassium chloride.

Hence in these two experiments, unfortunately carried out in the same year and situated in the same field, an additional ploughing, either in the autumn as well as in the spring or in the spring as well as in the autumn, did not increase the yield either of potatoes or of sugar-beet, except that it may have increased the efficiency of sodium chloride as a sugar-beet fertilizer.

### (C) *Heavy roll on the seed bed*

A field observation was made at Rothamsted several years ago which indicated that sugar-beet showed improved germination on a small area of land that had become heavily compacted. Two experiments on sugar-beet were thus set up to see if compacting the seed bed would in the normal course of events improve the germination and thus by leaving fewer gaps lead to a more uniform plant after singling. The compacting was carried out by a heavy roll. The results of the experiments are given in Table XVII.

Table XVII. *Effect of heavily compacting the seed bed on the yield of sugar-beet*

| | Increase of heavily rolled seed bed over ordinary rolled | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Spacing or rows apart | | | Sulphate of ammonia | | | | |
| | 10 in. | 15 in. | 20 in. | Absent | Present | Mean | S.E. of mean | Mean yield |
| Year 1934: | | | | | | | | |
| Roots (tons/acre) | −1·32 | −0·25 | 0·23 | −1·10 | 0·20 | −0·45 | 0·32 | 14·03 |
| Tops (tons/acre) | −0·82 | −0·78 | 0·02 | −0·79 | −0·26 | −0·53 | 0·35 | 11·62 |
| Sugar (cwt./acre) | −4·0 | 0·0 | 0·3 | −3·7 | 1·2 | −1·2 | 1·08 | 47·8 |
| Plant nos. (thousands/acre) | 4·1 | 2·1 | 2·9 | 3·2 | 2·9 | 3·0 | 0·85 | 47·9 |
| Year 1935: | | | | | | | | |
| Roots (tons/acre) | — | — | — | — | — | −0·22 | 0·20 | 11·57 |
| Tops (tons/acre) | — | — | — | — | — | −0·64 | 0·27 | 9·58 |
| Sugar (cwt./acre) | — | — | — | — | — | −0·4 | 0·68 | 39·5 |
| Plant nos. (thousands/acre) | — | — | — | — | — | −0·5 | 0·51 | 29·4 |

Heavy rolling thus did not lead to any improvement in the yield of roots, tops, or sugar, and although it may have increased plant numbers somewhat in 1934 when plant numbers were high, it led to no improvement in 1935 when they were low. But in 1934, taken in conjunction with the manurial dressings and the spacing of rows, heavy rolling does seem to have been definitely harmful with the narrow spacing, and also in the absence of a nitrogenous top dressing, and to have had no effect with a wide spacing or with a nitrogenous top dressing. Dung had no

influence on the response. In 1935 neither spacing nor the effect of sulphate of ammonia was included though the effect of the presence or absence of dung, agricultural salt and intensive inter-drill cultivation was included. None of these affected the response of the sugar-beet to rolling. Hence merely using a heavy roll on the sugar-beet seed bed has not had any great influence on the yield.

This result is in conformity with other published data on the effect of compacting the worked seed bed; von Nitzsch (1936), working in Germany, found that rolling the seed bed usually depressed the yield either of sugar-beet or mangolds and the depression was greater the heavier the roll used; Davies & Smyth-Homewood (1936*a*) at Wye and Culpin (1937) at Cambridge have both used a heavy roll after the land was ploughed and neither was able to find any great benefit from the consolidation, although Davies (1934) found in one year consolidation in strips, due to tractor wheels, produced an increase in yield that was nearly significant. But these negative results cannot be dismissed lightly, for compacting the subsoil with a furrow press is stated to be desirable on some soils and the tracks of tractor wheels are sometimes shown up in cereal crops by strips of better growth. Twice at Rothamsted, once on wheat and once on barley, dark and light strips have been seen on fields, and Davies (1932) and Davies & Smyth-Homewood (1936*b*) have given yields and photographs to illustrate this effect. Thus at Wye in 1931 the yield of spring-sown barley was nearly twice as large on the tractor tracks as between them. The effect occurs erratically and it has so far only been reported on poor land. It appears in fact to be equivalent to a top dressing of nitrogen on nitrogen-starved land. The cause is not yet known. Nor is it yet known whether the benefit is due to a compaction of the surface soil before ploughing or of the subsoil by the weight of the tractor wheels. The infrequent and haphazard appearance of this effect suggests that the compaction benefits the soil only under very restricted conditions. There does seem to be a possibility of useful results emerging if this effect can be studied in detail whenever it chances to appear.

## III. Cultivations during crop growth

### (A) *Rolling and harrowing winter wheat in spring*

Two experiments have been made on the effect of spring rolling and harrowing of winter wheat. In each case they have been superimposed on seed-bed cultivation experiments. In the 1931 experiments the main

plots were either ploughed, ploughed with the pulverator or cultivated with a Rototiller. In the 1933 experiment spring cultivation was superimposed on seed beds prepared by using either the plough or Rototiller working either deep or shallow, and on this was superimposed either the presence or absence of a top dressing of sulphate of ammonia. There were no important interactions between the spring and autumn cultivations or the nitrogenous top dressing. Table XVII shows, both for the 1931 and the 1933 experiment, the increase of yield due to harrowing (H), rolling (R) and harrowing and rolling (HR) over the control plots (O) that were neither rolled nor harrowed.

Table XVIII. *Increase in yield of winter wheat due to rolling or harrowing in the spring*

| | | Increase in yield (cwt./acre) | | | | |
|---|---|---|---|---|---|---|
| | | H–O | R–O | HR–O | S.E. | Mean yield |
| Year 1931 | Grain | 1·8 | 0·8 | 2·1 | 0·57 | 15·8 |
| | Straw | −0·2 | 3·0 | −0·7 | 1·32 | 39·1 |
| Year 1933 | Grain | 0·3 | 0·3 | 1·4 | 1·1 | 23·3 |
| | Straw | −0·7 | 1·8 | −0·6 | 2·1 | 34·0 |

The same main results were found in each experiment: combined rolling and harrowing increased the yield of grain probably more than either implement used alone; the harrow may increase the yield of grain more than the roll; rolling alone tended to increase the yield of straw, while the harrow used either alone or with the roll reduced the yield of straw.

Results obtained elsewhere in this country on the whole fall into line with these. Out of the eight experiments done on the Cambridge University Farm (Garner & Sanders, 1937) during the five harvest years 1930–6 on both heavy and light land, the spring cultivations of either rolling or harrowing or both together often increased the grain yield by about 2 % and had no effect on the straw yield. But in 1930–1 combined rolling and harrowing appeared to be very efficacious on both the heavy and light soil, increasing the grain yield by about 10 %, which was as a matter of fact significant only on the heavy land, and the straw yield by 5 % which was not significant on either soil. Unfortunately in this year the effects of the roll alone or harrows alone could not be studied. The Cambridge workers (Sanders, 1935; Culpin, 1937) have also published two experiments on light chalky soil and obtained in each case a significant increase for the yield of grain due to spring rolling. Sanders pointed out that in the first of these experiments the spring cultivation was able

to exert a strong depressing effect on the growth of poppies, the dominant weed in the wheat on this farm. At Wye a note in the farm report (Davies & Smyth-Homewood, 1937) states that an experiment similar to the above was made in 1935–6 and that the spring cultivations had no effect.

The main results therefore from the English experiments are that spring cultivations have never reduced the yield of grain; they may give an increase of 1–2 cwt./acre of grain; and they may help to control weeds if carried out at the right time.

### (B) *Inter-drill hoeing of root crops*

It is a common statement in books and articles on the growing of sugar-beet that the hoes should be kept moving as long as possible to keep down weeds and to preserve a fine mulch between the plants. Experiments have been carried out at Rothamsted for the three years 1932, 1934, 1935 and at Woburn on a light sandy soil in 1932 to test this point. The experiment, which was always superimposed on a manurial experiment, consisted in hoeing some plots sufficiently to keep

Table XIX. *Influence of intensive inter-drill hoeing over normal hoeing on the yield of sugar-beet*

| | Increase of intensive over normal hoeing | | |
|---|---|---|---|
| | Mean | S.E. of mean | Mean yield |
| Rothamsted 1932: | | | |
| Roots (tons/acre) | −1·03 | 0·14 | 13·41 |
| Tops (tons/acre) | −2·56 | 0·35 | 14·58 |
| Sugar (cwt./acre) | −4·0 | 0·51 | 50·1 |
| No. of hoeings, normal 3, extra for intensive 5. | | | |
| Rothamsted 1934: | | | |
| Roots (tons/acre) | −1·79 | 0·27 | 15·36 |
| Tops (tons/acre) | −0·53 | 0·31 | 14·36 |
| Sugar (cwt./acre) | −7·2 | 0·95 | 54·5 |
| Plant no. (thousands/acre) | −0·1 | 0·39 | 30·9 |
| No. of hoeings, normal 2, extra for intensive 6. | | | |
| Rothamsted 1935: | | | |
| Roots (tons/acre) | −0·25 | 0·20 | 11·57 |
| Tops (tons/acre) | 0·26 | 0·27 | 9·58 |
| Sugar (cwt./acre) | 1·0 | 0·68 | 39·5 |
| Plant no. (thousands/acre) | −0·3 | 0·51 | 29·4 |
| No. of hoeings, normal 3, extra for intensive 5. | | | |
| Woburn 1932: | | | |
| Roots (tons/acre) | −0·23 | 0·21 | 11·88 |
| Tops (tons/acre) | 0·52 | 0·26 | 15·80 |
| Sugar (cwt./acre) | −1·3 | 0·75 | 43·0 |
| No. of hoeings, normal 5, extra for intensive 3. | | | |

down the worst weeds and in hoeing others at approximately fortnightly intervals throughout the summer. The results of these experiments are given in Table XIX. Only the means are given, in no case was there any effect of the manurial or other experimental treatment on the effect of intensive inter-drill hoeing.

These experiments therefore lend no support to the idea that yields are increased by extra hoeings. On the contrary they show the sole reward for the extra cost and labour involved is either no significant increase in yield or else an actual, and sometimes a serious, depression. The possibility cannot of course be entirely dismissed that these extra cultivations may have caused some damage to the leaves and superficial roots of the plants, which may have more than offset any beneficial effect of the cultivations themselves on the soil fertility. The damage, if any, was insufficient to affect plant numbers appreciably. The work was carried out by skilled and not by casual labour, so that any damage occurring would be inherent in the operation and not due to lack of skill.

A further experiment was done on kale at Rothamsted in 1932, with the following results:

| | Increase of intensive over normal hoeings | | |
|---|---|---|---|
| | Mean | S.E. of mean | Mean yield |
| Green weight (tons/acre) | −1·84 | 0·32 | 25·5 |

No. of hoeings, normal 2, extra for intensive 5.

This experiment too gave a significant depression of yield for the extra hoeings.

The conclusion from these experiments is thus that hoeings beyond the modest minimum required to keep down the worst weeds and to stop too hard a cap forming on soil prone to this behaviour is useless labour at the best, and may even reduce the crop yield.

## Conclusions

The outstanding conclusiou reached from these experiments is the wide freedom a farmer appears to possess in choosing what implements to use or what operations to carry out. On the whole the numerous experiments carried out here and elsewhere in this country show that yields are not greatly dependent on cultivation methods, though some of the time-honoured methods are good stabilizers of yield, that is they give a smaller chance of poor yields. But if speed of operation becomes important, as it may do in rather broken weather, it will often be better

to use a rapid method of cultivation that does not appear to do the work so well as the traditional one, and the yield would probably not be seriously reduced. There is another obvious application. For example, when a seed bed is being prepared in dry weather it is not desirable to cultivate the bed so frequently or so well that it is dried out. A hasty cultivation before drilling will conserve the moisture far better than a thorough cultivation and will thus encourage germination.

Although we believe that our results are, in general, directly applicable to farming practice, we fully realize that convinced exponents of thorough and intensive cultivation can legitimately criticize them on at least three points: that the experiments were done on a restricted type of soil, that the operations were not all carried out at the most suitable times, and that cultivation is an art and therefore not capable of exact and scientific treatment. A short discussion of these points is given below.

It is possible that these negative results are only found on restricted types of soil, restricted either because the particular soil does not respond well to cultivation or because it has been so well cultivated in the past that liberties can be taken with it now or because it is in a high state of fertility. Until the experiments have been carried out on a much larger range of soil types it is impossible to reply to this criticism. It is worth while, however, pointing out that certain types of crops, particularly long term grass or leguminous leys, are generally believed to improve the structure of the soil so markedly that such soils need very mnch less cultivation than others on which these crops are rarely grown. It is possible that the art of increasing crop yields by appropriate cultivations had most scope in the priuitive farming before manures and leys were in general use, and it may well be that cultivations are less important the higher the farming and the higher the fertility of the land.

The second criticism is that cultivations only markedly improve crop yields if carried out under restricted soil or plant conditious, and that if the right day is missed the effect of the cultivation on another day will be negligible. If really beneficial effects can only be obtained under such restricted conditions these cultivations are naturally of very little interest to those mechanized farmers who are working as large an area of land as possible with the minimum number of tractors, men, and implements, for in such a variable climate as this country possesses they would rarely have time to take advantage of such very transient conditions over their whole farm.

This criticism is, however, very important. It is important to use

an implement only when conditions are suitable and it is better not to cultivate than to cultivate at the wrong time. It is quite possible that the reason so many negative results were obtained on this farm is that the operations were not always carried out at the correct times. But the farm manager of the experimental farm here and of those elsewhere are competent farmers, and they choose the day on which the cultivations are to be done. If they cannot recognize the exact conditions under which these cultivations will be markedly beneficial it is certain that most farmers in this country will also fail to recognize them.

A fundamental trouble in trying to determine these conditions is that it is not yet known what cultivation operations are really intended to do. Their main visual results can be described but there is no generally recognized method of assessing the agricultural usefulness of any particular operation. Attempts to do this have often been made but so far only one method is claimed to be fairly successful for conditions prevalent in this country. In Germany von Nitzsch, under the auspices of the Government Department for Agricultural Technology (Reichskuratorium für Technik in der Landwirtschaft), claims that on the whole the fundamental usefulness of a cultivation operation can be measured by the increase of the soil pore-space brought about during the growing season. He states (1936) that for every 1 % the pore-space can be increased during the growing season the yield is increased by 2·5–3 % and that increases of over 3 % in pore space or 10 % in yield are not difficult to obtain.

The third criticism of cultivation experiments sometimes made is that cultivation is an art, and therefore, unlike a science, is not susceptible to experimentation. The philosophical implications of this criticism can be left for the future, but it is possible that the experimental methods employed may not be entirely suited for determining the effect of cultivations. No experimenter would think of asserting that the technique of carrying out cultivation experiments is anywhere near perfect, and indeed one of the fundamental technical difficulties of seed-bed cultivation experiments has already been pointed out. But the fact remains that the experiments simulate the conditions prevailing on a very large number of farms and it is unlikely that any of the results would be much different if carried out by commercial farmers on the same land.

SUMMARY

1. Wheat, barley or mangolds gave nearly the same yield if grown on seed beds prepared by ploughing and harrowing, by using the grubber or cultivator and harrowing and by using the Rototiller, provided that the cultivators were used for one year only. If used for several years in succession deterioration of yield sometimes sets in, possibly due to the increased weediness of the non-ploughed plots.

2. There was no advantage in ploughing deeper than 4 in. but it is advantageous to use the grubber or Rototiller deeper.

3. The effect of cross-ploughing, subsoiling or heavy rolling the seed bed for spring-sown crops was without effect on the yield.

4. Spring rolling and harrowing improved the yield of winter wheat but had little effect on the yield of straw. Rolling alone may have produced a slightly increased yield of grain but it improved the straw yield while harrowing depressed the straw yield.

5. There was strong evidence that intensive hoeing of sugar-beet or kale is detrimental. Two to three hoeings appear to be ample.

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# AN ATTEMPTED FRACTIONATION OF THE SOIL PHOSPHORUS

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For over a century attempts have been made to estimate the available phosphoric acid in soils by determining the amount dissolved by dilute or weak acids, and less frequently by salts and alkalis. Generally there was no evidence that the methods proposed completely extracted a definite group of compounds. The results were often greatly complicated by reactions between the acids and the non-phosphatic soil constituents; thus with dilute solutions of acids a large proportion of the total acid might be neutralized by calcium carbonate, and with acid soils it was often found that the amount of phosphorus dissolved diminished with time through precipitation by activated sesquioxides. Many of the extraction methods have been intended to reproduce conditions somewhat analogous to the processes which were believed to proceed near the roots of plants. An alternative method would be to aim at a definite fractionation of the soil phosphorus compounds and then to endeavour to interpret the availability of these various fractions by considering the general composition and environmental conditions of the soil. Such a fractionation would have to be reasonably complete in the sense that a repetition of the extractions or minor modifications in the technique would not seriously affect the amount of a given fraction. If possible the fractions should be related to known materials.

Russell (1932) grouped the phosphorus compounds occurring in soils into:

(*a*) Inorganic phosphorus in neutral soils: probably a calcium phosphate: hydroxyapatite.

(*b*) Inorganic phosphorus in acid soils: presumably combinations with iron and aluminium oxides.

(*c*) Organic phosphorus compounds.

It is known that solutions sufficiently acid to dissolve calcium phosphates, such as hydroxyapatite, also dissolve appreciable amounts of iron phosphates, and that hydroxy-acids, such as citric, dissolve iron

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phosphate readily. Potassium carbonate has been used empirically with some success by Das (1930) and Hockensmith *et al.* (1933) to determine the availability of phosphates in highly calcareous soils where the usual acid extraction methods were unsuitable. In the present paper an attempt was made to combine both an acid and an alkaline extraction for the purpose of fractionating the soil phosphorus.

## SOLUBILITY OF PHOSPHATIC MATERIALS IN SODIUM HYDROXIDE AND SODIUM CARBONATE

It became necessary at the outset to determine the solubility of various phosphatic materials in alkaline solutions. Amounts of material containing 10 mg. P were digested at 95° C. overnight with either 0·25 *N* sodium hydroxide or 0·2 *N* sodium carbonate. The solutions were diluted to 500 c.c., allowed to stand overnight and suitable aliquots of supernatant liquid analysed. The extractions of the three calcium phosphates were also done in the presence of 1 g. of calcium acetate in order to determine the effect of a considerable excess of calcium (Table I).

Table I. *Solubilities of phosphatic materials in sodium hydroxide and sodium carbonate solutions*

| Materials used | Origin | mg. P taken | % total P soluble in 0·2 *N* $Na_2CO_3$ | % total P soluble in 0·25 *N* NaOH |
|---|---|---|---|---|
| Monocalcium phosphate | *a* | 10 | 78 | 73 |
| Dicalcium phosphate | *a* | 10 | 38 | 28 |
| Tricalcium phosphate | *a* | 10 | 10 | 7 |
| Hydroxyapatite | *c* | 18·5 | 4 | 1 |
| Monocalcium phosphate and 1 g. calcium acetate | *a* | 10 | 20 | 0·5 |
| Dicalcium phosphate and 1 g. calcium acetate | *a* | 10 | 13 | 0·2 |
| Tricalcium phosphate and 1 g. calcium acetate | *a* | 10 | 4 | 0·2 |
| Ferric phosphate $Fe_2O_3.P_2O_5.2H_2O$ (special laboratory preparation) | *d* | 10 | 98 | 99 |
| Dufrenite $2Fe_2O_3.P_2O_5.3H_2O$ | *e* | 10 | 99 | 99 |
| Delvauxite $2Fe_2O_3.P_2O_5.xH_2O$ | *f* | 10 | 88 | 88 |
| Aluminium phosphate (B.F.H.) | *b* | 10 | 48 | 100 |

(*a*) Purchased from Kahlbaum "Für Analyse". (*b*) Purchased from British Drug Houses.
(*c*) Prepared by G. Trömel. (*d*) Prepared by L. M. Weyker.
(*e*) Purchased from Ward's Natural Science Establishment. (*f*) Supplied by the British Natural History Museum, South Kensington.

In the sodium carbonate solution, which ensured a low calcium concentration, the solubility of the calcium phosphates decreased rapidly with increasing basicity. The addition of a large excess of calcium acetate reduced the solubility of the mono-, di- and tricalcium phosphates to about one-third.

In the sodium hydroxide solution, which allowed more of the calcium from the phosphates to remain in solution, the solubilities of the mono-, di- and tricalcium phosphates were slightly lower and that of the hydroxyapatite much lower than in the sodium carbonate solution. In the presence of an excess of calcium all the calcium phosphates had very low solubilities in sodium hydroxide. The iron and aluminium phosphates were almost completely hydrolysed by both alkaline solutions and their phosphorus was dissolved.

### Soil phosphorus soluble in sodium hydroxide

Overnight 5 g. of soil were digested with 100 c.c. of 0·25 *N* sodium hydroxide at 95° C. The total alkali-soluble phosphorus was determined on an aliquot which was evaporated with magnesium nitrate and ignited.

An analytical procedure for the separation of the inorganic from the organic phosphorus in the alkaline extract was studied. Trials showed that the coloured extracts could be decolorized, thus facilitating a colorimetric determination of the free phosphate ions or inorganic phosphorus by Denige's method (Truog & Meyer, 1929) by acidifying, filtering off the humus and shaking the filtrate with kieselguhr. Results using this technique showed that a considerable portion of the total phosphorus in the above extract was not present as free phosphate ions. This would point towards the existence of an organic phosphorus fraction. Because this kieselguhr method was tedious and subject to the objections that there were losses of phosphorus by absorption, the possibility of using a bromine oxidation similar to that suggested by Hockensmith *et al.* (1933) was investigated. A comparison of the results obtained, using the kieselguhr and the bromine methods on extracts from widely different soils showed substantial agreement; the mean difference was $-2·6 \pm 3·6$ (Table II). Consequently it was concluded that there were definite organic and inorganic fractions in the alkali extract. The amount of phosphorus determined colorimetrically on the decolorized extract was assumed to be inorganic, and the remaining phosphorus was assumed to be organic.

The organic and inorganic sodium hydroxide-soluble phosphorus was determined on thirty-four widely different soils from Great Britain, Russia, Africa, continental United States and Hawaii (Appendix I). The statistical analyses (Appendix II B) showed that the alkali-soluble inorganic phosphorus decreased significantly with the *p*H, at the rate of 7–8 % per unit increase in *p*H. There was also some barely significant

evidence that this percentage also decreased as the carbon increased. The organic phosphorus increased significantly with the carbon contents of the soils. In soils of equal carbon content there was a tendency for acid ones to have a higher organic phosphorus content.

Table II. *Comparison of the kieselguhr and bromine methods of determining the inorganic alkali-soluble phosphorus*

| | p.p.m. inorganic phosphorus | | |
|---|---|---|---|
| Soil | Kieselguhr method | Bromine method | Difference |
| 1438 | 512 | 500 | -12 |
| 1440 | 45 | 56 | 11 |
| 1441 | 145 | 150 | 5 |
| 1442 | 150 | 156 | 6 |
| 1443 | 588 | 612 | 24 |
| 1444 | 230 | 225 | - 5 |
| 1445 | 20 | 25 | 5 |
| 3333 | 100 | 105 | 5 |
| 3334 | 110 | 105 | - 5 |
| 3335 | 85 | 85 | 0 |
| 3330 | 825 | 825 | 0 |
| 3331 | 210 | 175 | -35 |
| 3329 | 650 | 625 | -25 |
| 3336 | 280 | 263 | -17 |
| 2865 | 35 | 40 | 5 |
| 2899 | 35 | 32 | - 3 |
| Mean | 251 | 249 | - 2·56 |

## THE EFFECT OF ACTIVE SOIL CALCIUM ON THE SODIUM HYDROXIDE-SOLUBLE PHOSPHORUS

Since the soil reaction appeared to be related to both the organic and inorganic sodium hydroxide-soluble phosphorus, it seemed possible that the active soil bases, such as calcium and magnesium, might be factors in this relationship. Some of the phosphorus liberated by the sodium hydroxide might be reprecipitated by calcium. The soil samples were therefore treated with sodium acetate to remove exchangeable calcium and the alkali-soluble phosphorus determined on these sodium-saturated soils. (The results are reported in Appendices I and II.)

The relationship between the inorganic phosphorus and *p*H was no longer significant, but it became significant if corrections were made for the differences in carbon and clay contents, i.e. the partial regression coefficient was significant in the multiple regression equation on *p*H, carbon and clay. The sodium acetate pre-treatment caused large increases in the amounts of inorganic phosphorus extracted from many of the neutral and calcareous soils, but often decreased the amount from acid soils. The amount of change in the inorganic alkali-soluble phosphorus

resulting from this pre-treatment with sodium acetate showed a significant relation to the *p*H of the original soils.

The pre-treatment with sodium acetate caused the amount of organic phosphorus to be increased by nearly one-half. The relation between the organic phosphorus and carbon still remained highly significant but the effect of *p*H was no longer significant.

The increase in inorganic alkali-soluble phosphorus in neutral soils through pre-treatment with sodium acetate suggests that the distinction between acid and neutral soils depends primarily on the calcium brought into solution. The evidence also strongly indicates that the amount of organic phosphorus extracted from soils by sodium hydroxide depends on the calcium concentration.

### Acid-soluble phosphorus remaining after the sodium hydroxide extraction

Extracting a soil with sodium hydroxide presumably left in the soil basic calcium phosphates, which were estimated by extracting the treated soils with 0·5 *N* sulphuric acid. For samples extracted directly with sodium hydroxide without prior treatment with sodium acetate, the acid-soluble phosphorus was found to increase by 8 % of the total per unit increase in soil *p*H, the effect being highly significant. It is probable that some of the phosphorus dissolved by the sulphuric acid was from compounds formed by an interaction of the soil calcium with the phosphorus set free during the alkali extraction. An attempt to eliminate this was made by subtracting from the acid-soluble phosphorus *the difference* between the values for alkali-soluble phosphorus determined (*a*) directly, and (*b*) after previous treatment with sodium acetate. The validity of this correction depends upon the assumption that the sum of the acid- and alkali-soluble inorganic phosphorus is a constant (see p. 239). The adjusted values of the acid-soluble phosphorus were not significantly related to soil *p*H values, but the general tendency was for neutral soils to have more acid-soluhle phosphorus than acid ones.

### Phosphorus insoluble in sodium hydroxide and sulphuric acid

When the sum of the inorganic alkali-soluble, inorganic acid-soluble (both without NaAc pre-treatment) and organic phosphorus (with NaAc pre-treatment) is subtracted from the total phosphorus, a large percentage of the total still remains unaccounted for. In order to determine

whether this insoluble fraction was a definite one, or merely the result of incomplete extraction of the inorganic phosphorus, the following different series of repeated extractions were compared:

(A) 0·25 $N$ NaOH followed by two 0·5 $N$ $H_2SO_4$ extractions.

(B) Two 0·25 $N$ NaOH extractions followed by one 0·5 $N$ $H_2SO_4$ extraction.

(C) 0·5 $N$ $H_2SO_4$ followed by 0·25 $N$ NaOH followed by 0·5 $N$ $H_2SO_4$.

The results (Table III) show that additional extractions above those originally proposed do not increase the total amount extracted to any marked degree, indicating that this insoluble portion of the total phosphorus is definitely inert.

Table III. *Phosphorus fractions in parts per million with repeated extractions in different orders*

| Soil no. ... | 1438 | 1444 | 1314 | 1330 | 2935 | 3334 | 3335 | 3329 |
|---|---|---|---|---|---|---|---|---|
| Treatment: | | | | | | | | |
| A NaOH | 500 | 180 | 65 | 70 | 60 | 105 | 75 | 525 |
| A $H_2SO_4$ | 950 | 50 | 145 | 240 | 515 | 50 | 105 | 75 |
| A $H_2SO_4$ | Trace | Trace | Trace | Trace | Trace | Trace | Trace | Trace |
| B NaOH | 500 | 165 | 65 | 65 | 60 | 105 | 75 | 450 |
| B NaOH | 15 | 5 | 25 | 25 | 25 | 5 | 20 | 130 |
| B $H_2SO_4$ | 900 | 30 | 135 | 230 | 550 | 40 | 105 | 25 |
| C $H_2SO_4$ | 1190 | 180 | 125 | 280 | 550 | 100 | 145 | 45 |
| C NaOH | 215 | 55 | 35 | 40 | 130 | 50 | 65 | 440 |
| C $H_2SO_4$ | 20 | 1 | 1 | 1 | Trace | 1 | 1 | 10 |
| Totals: | | | | | | | | |
| A | 1450 | 230 | 210 | 310 | 575 | 150 | 180 | 600 |
| B | 1415 | 200 | 225 | 320 | 635 | 150 | 200 | 605 |
| C | 1425 | 236 | 161 | 321 | 680 | 151 | 211 | 495 |
| Total P in soil | 2750 | 700 | 750 | 800 | 1300 | 350 | 550 | 2000 |

The insoluble phosphorus was the largest single fraction, the mean for the thirty-four mineral soils was 43·2 % of the total phosphorus. The proportion of insoluble phosphorus decreased as the proportion of organic phosphorus and carbon increased.

## Phosphorus by Truog's method

The Truog (1930) method for determining the readily available phosphorus by means of 0·002 $N$ $H_2SO_4$ buffered to $p$H 3 was used. On the thirty-four mineral soils the amount of phosphorus thus determined increased significantly at the rate of 4 % of the total for unit increase in $p$H.

THE DISTRIBUTION OF PHOSPHORUS IN THE CLASSICAL PLOTS AT ROTHAMSTED AND WOBURN

The classical continuous wheat plots at Rothamsted and barley plots at Woburn offer an excellent opportunity for studying the residual effects of continuous applications of superphosphate and dung. These soils have different degrees of acidity, the Rothamsted soil being a slightly calcareous clay loam (*p*H 7·4–8·0) and the Woburn soil an acid sandy loam (*p*H 4·8–6·0). The mineral manure plots at Rothamsted received 3·5 cwt. of superphosphate per acre annually since 1843. The dunged plot received annually 14 tons of farmyard manure per acre containing approximately the same amount of phosphorus as was given to the mineral manure plots. At Woburn the annual dressing was 3·5 cwt. of superphosphate per acre for the first 30 years and 3·0 cwt. per acre for the next 20 years. The dunged plot received an annual farmyard manure dressing which contained an amount of phosphorus approximately equivalent to 2·5 cwt. of superphosphate per acre.

Two sets of soil samples taken with an interval of 45 years between them at both Rothamsted and Woburn were analysed by the proposed system of fractionating the soil phosphorus. The results in Table IV for the unmanured, minerals only, minerals and sulphate of ammonia, and dung plots show distinct differences in the distribution of the phosphorus in the two soil types. The Rothamsted soil had larger amounts of acid-soluble phosphorus, and the Woburn soil larger amounts of alkali-soluble phosphorus. This relationship held true after allowing for the calcium effect. The insoluble fraction remained almost unchanged, showing that the phosphorus applied to the soils as fertilizers remained only in forms soluble in the alkali and acid treatments. The influence of phosphorus fertilization on the organic phosphorus was not very definitely shown, but there was an indication that when both phosphorus and nitrogen were present in abundance and the crop yields were high in consequence, there was a greater accumulation of organic phosphorus.

METHODS OF ANALYSIS

*Sodium hydroxide extract.* Overnight 5 g. of soil were digested at 95° C. with 100 c.c. 0·25 *N* NaOH, the suspension transferred to a 500 c.c. volumetric flask, made to volume and allowed to stand 24 hours.

*Kieselguhr decolorization of the* NaOH *extract.* To a 50 c.c. aliquot of supernatant alkali extract 2 c.c. of 6 *N* $H_2SO_4$ were added and the solution heated until the precipitated humus coagulated. This solution was then filtered into a 100 c.c. volumetric flask; the paper washed with

Table IV. *Distribution of phosphorus in parts per million in continuous plots at Rothamsted and Woburn*

| | pH | Carbon % | Total P | Inorganic acid-soluble | Inorganic alkali-soluble | Organic alkali-soluble after NaAc treatment | Insoluble | Truog acid-soluble | Inorganic alkali-soluble after NaAc treatment | Estimated inorganic acid-soluble after NaAc treatment |
|---|---|---|---|---|---|---|---|---|---|---|
| **Rothamsted continuous wheat** | | | | | | | | | | |
| **1893 samples:** | | | | | | | | | | |
| Plot 3 No manure | 8·1 | 0·9 | 600 | 140 | 40 | 80 | 340 | 10 | 80 | 100 |
| 5 Minerals | 8·3 | 0·9 | 1050 | 500 | 40 | 70 | 440 | 100 | 70 | 470 |
| 8 Minerals and sulphate of ammonia | 8·1 | 1·1 | 950 | 365 | 40 | 100 | 445 | 110 | 140 | 265 |
| 2*b* Dung | 7·5 | 2·3 | 950 | 375 | 50 | 100 | 425 | 95 | 90 | 335 |
| **1936 samples:** | | | | | | | | | | |
| Plot 3 No manure | 7·7 | 1·1 | 600 | 175 | 30 | 90 | 305 | 21 | 80 | 125 |
| 5 Minerals | 8·0 | 1·0 | 1250 | 735 | 40 | 75 | 400 | 270 | 170 | 605 |
| 8 Minerals and sulphate of ammonia | 7·7 | 1·2 | 1150 | 575 | 40 | 225 | 310 | 270 | 300 | 315 |
| 2*b* Dung | 7·4 | 2·7 | 1300 | 565 | 55 | 250 | 430 | 310 | 225 | 395 |
| **Woburn continuous barley** | | | | | | | | | | |
| **1888 samples:** | | | | | | | | | | |
| Plot 1 No manure | 5·8 | 1·3 | 700 | 105 | 125 | 185 | 335 | 20 | 205 | 25 |
| 4 Minerals | 6·0 | 1·2 | 800 | 110 | 225 | 185 | 280 | 44 | 290 | 45 |
| 5 Minerals and sulphate of ammonia | 5·5 | 1·2 | 800 | 110 | 225 | 195 | 270 | 34 | 280 | 65 |
| 11*b* Dung | 6·1 | 1·5 | 800 | 145 | 145 | 220 | 290 | 34 | 255 | 35 |
| **1927 samples:** | | | | | | | | | | |
| Plot 1 No manure | 5·5 | 0·9 | 700 | 50 | 175 | 200 | 275 | 12 | 150 | 75 |
| 4 Minerals | 6·0 | 0·9 | 1100 | 160 | 315 | 250 | 375 | 82 | 325 | 150 |
| 5 Minerals and sulphate of ammonia | 4·8 | 1·0 | 1100 | 105 | 475 | 280 | 240 | 48 | 385 | 195 |
| 11*b* Dung | 5·8 | 1·5 | 1050 | 100 | 275 | 310 | 355 | 46 | 290 | 85 |
| Average of eight Rothamsted soils | 7·9 | 1·4 | 937 | 430 | 42 | 124 | 387 | 148 | 144 | 326 |
| Average of eight Woburn soils | 5·7 | 1·2 | 871 | 110 | 282 | 228 | 302 | 40 | 297 | 84 |

hot water and the filtrate made to volume. A portion of the filtrate was shaken a few minutes with kieselguhr (acid washed) and filtered.

*Bromine decolorization of the* NaOH *extract*. An aliquot (about 10 c.c.) of the supernatant alkali extract was placed in a 100 c.c. flask, 5 c.c. bromine water added, the solution boiled until the free bromine was expelled, more bromine being added if the decolorization was not complete. Five drops of 6 $N$ $H_2SO_4$ were then added and the free bromine boiled off. The cooled solution was filtered.

*Total* NaOH-*soluble phosphorus*. A 10 c.c. aliquot of extract was evaporated with 2 c.c. of 10% $Mg(NO_3)_2$, ignited, the residue dissolved by digesting with 25 c.c. 0·5 $N$ $H_2SO_4$ and the phosphorus determined on an aliquot.

*Acid-soluble phosphorus remaining after the* NaOH *extraction*. The soil after extraction with NaOH was used in this determination. All but 100 c.c. of the supernatant liquid in the volumetric flask was siphoned off and discarded. The remaining suspension was carefully neutralized to $p$H 7·0 with $N$ HCl using brom thymol blue as an outside indicator. The suspension was then filtered on a Büchner funnel, the soil washed with neutral 0·5 NaCl, the soil and filter paper transferred to a suitable bottle and shaken 1 hr. with 250 c.c. of 0·5 $N$ $H_2SO_4$. The acid-soluble phosphorus was determined on an aliquot of the filtered extract.

*Total soil phosphorus*. The total soil phosphorus was determined by fusing the soil with sodium carbonate and extracting the melt with water.

*Phosphorus determination*. All phosphorus determinations were made colorimetrically by Truog & Meyer's (1929) modification of the Denige method.

*Carbon*. The carbon was determined by the method of Walkley (1935).

*p*H. The $p$H was determined colorimetrically by the method of Kuhn (1930).

## Discussion

The foregoing experiments have shown that by extraction with sodium hydroxide, followed by an acid, it is possible to divide the phosphorus compounds of soils into three broad fractions, viz.:

(1) Organic compounds soluble in sodium hydroxide.

(2) Inorganic compounds dissolved by extraction with sodium hydroxide followed by an acid.

(3) Insoluble compounds.

It was found convenient to express the phosphorus fractions as a percentage of total phosphorus, thus making possible a comparison of soils of widely different total phosphorus contents.

It was seen that in order to obtain a complete extraction of the organic phosphorus it was necessary first to saturate the soils with sodium and remove the replaceable calcium. On the average of the thirty-four mineral soils after removing active calcium about 60 % of the alkali-soluble phosphorus was organic. This organic phosphorus in soils is interesting since a mild bromine oxidation will not cause its decomposition into inorganic phosphorus. Shorey (1913), Auten (1923), and others have suggested that the organic phosphates of soils are mostly nucleic acids and their derivatives. When nucleic acids are oxidized under some conditions they are converted into nucleotides and no inorganic phosphorus is liberated. This may explain why the organic phosphorus in the sodium hydroxide extracts resisted oxidation by bromine. The fact that organic phosphorus in mineral soils is directly related to the carbon or organic matter content may be taken to imply that the organic phosphorus is mainly a function of plant and biological activities.

A study of the solubility of inorganic phosphatic materials in sodium hydroxide revealed that tricalcium phosphate and apatite were relatively insoluble, while iron, aluminium, mono- and dicalcium phosphates were soluble. Further, the presence of free calcium in solution caused a marked depressing of the solubility of the otherwise soluble phosphorus. The exchangeable calcium had a marked effect on the solubility of the inorganic phosphorus in sodium hydroxide, especially with neutral to calcareous soils. When these thirty-four different soils were first saturated with sodium and the inorganic soluble phosphorus determined, about 60 % was soluble in sodium hydroxide and 40 % in acid. It is interesting to note that all of the phosphorus applied to the continuous plots at Rothamsted and Woburn can be extracted by the combined alkali-acid treatment, and further, that the bulk of the phosphorus in the neutral Rothamsted soil is not alkali-soluble, whereas in the acid Woburn soil the bulk of the phosphorus is alkali-soluble. Reasoning by analogy, much of the phosphorus in the neutral soil appear to be present as tricalcium phosphate or apatite, and that in the acid soil as iron, aluminum or mono- and dicalcium phosphates.

The present work does not allow us to decide whether the soluble inorganic phosphorus in soils is present in association with the soil colloids or as a mosaic throughout the soil mass. However, the fact remains that many neutral to calcareous soils contain much phosphorus that is not present as apatite or hydroxyapatite and many acid soils contain phosphorus which is probably in the form of apatites or tricalcium phosphates. This would favour the mosaic theory.

The presence of an insoluble and relatively inert fraction of the soil phosphorus has been shown. The fact that this fraction did not change after over 50 years of fertilization at Rothamsted and Woburn indicated that it is relatively unimportant in problems of phosphate absorption. It is quite possible that this fraction may form a part of the clay lattice as suggested by Marshall (1935).

## Summary


1. Extractions of soils with sodium hydroxide, followed by an acid, have been used in an attempt to fractionate the soil phosphorus.

2. Colorimetric methods for the estimation of the organic and inorganic phosphorus in alkali soil extracts have been suggested.

3. The amount of soil phosphorus soluble in sodium hydroxide is affected by the active soil calcium. It is suggested that sodium-saturated soils be used when studying the alkali-soluble phosphorus.

4. The acid-soluble phosphorus remaining in soil after extraction with sodium hydroxide was determined. This fraction appears by analogy to be similar to the apatites.

5. The largest fraction of the total soil phosphorus was not dissolved by the sodium hydroxide and acid extractions. This fraction was not increased by the long-continued use of phosphatic fertilizers at Rothamsted and Woburn.

6. Relatively large amounts of organic phosphorus were found in soils and the amounts were closely related to the carbon contents.


## Acknowledgement


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# APPENDIX I

| Origin | Description | No. | Org. C % *a* | *p*H *b* | Total P p.p.m. *c* | Oven-dry clay % *y* | Inorg. alk.-sol. *i* | Org. alk.-sol. *o* | Inorg. acid-sol. *e* | Inorg. alk.-sol. *t* | Org. alk.-sol. *k* | Estimated acid-sol. *x* | In-soluble *m* | Truog *g* | Increase in alk.-sol. through NaAc *v* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Description, carbon %, *p*H, total phosphorus and clay content of thirty-six soils | | | | | | Fractions of soil phosphorus expressed as percentages of total phosphorus: Without NaAc | | | With NaAc | | | Other fractions | | |
| **Great Britain:** | | | | | | | | | | | | | | | |
| Rothamsted | (*a*) Broadbalk headland, heavy loam | 1441 | 2·0 | 5·6 | 900 | 18·0 | 17·2 | 19·4 | 10·6 | 23·9 | 31·7 | 3·9 | 40·6 | 1·9 | 6·7 |
| Rothamsted | Broadbalk Wilderness (grass) | 3338 | 3·8 | 7·4 | 750 | 21·3 | 7·3 | 18·7 | 19·2 | 17·3 | 32·0 | 9·3 | 41·3 | 7·5 | 10·0 |
| Rothamsted | Park Grass, Plot 14 | 3339 | 4·0 | 5·8 | 1550 | 20·5 | 14·8 | 23·4 | 35·2 | 40·3 | 32·2 | 9·7 | 17·8 | 9·7 | 25·5 |
| Woburn | (*a*) Light loam from Lower Greensand | 1442 | 0·9 | 5·9 | 700 | 10·3 | 17·8 | 17·0 | 7·1 | 23·1 | 20·7 | 2·8 | 54·3 | 1·9 | 4·3 |
| Harper Adams | (*a*) Light loam from Bunter Drift | 1444 | 0·9 | 4·9 | 700 | 7·7 | 25·7 | 14·3 | 7·1 | 21·4 | 22·9 | 11·4 | 44·3 | 4·9 | −4·3 |
| Chipping Norton | Red loam from Middle Lias | 3340 | 2·6 | 6·1 | 2550 | 25·0 | 20·2 | 14·7 | 4·6 | 22·6 | 22·9 | 2·3 | 52·2 | 5·5 | 2·4 |
| Tunstall | (*c*) Light blowing sand | 2899 | 0·5 | 7·0 | 300 | 2·6 | 11·7 | 4·3 | 16·7 | 28·3 | 20·0 | 0·1 | 51·6 | 13·3 | 16·6 |
| King's Lynn | (*c*) Sandy loam | 2865 | 1·1 | 8·0 | 650 | 9·9 | 5·4 | 3·8 | 29·2 | 14·6 | 16·9 | 20·0 | 48·5 | 14·8 | 9·2 |
| King's Lynn | (*c*) Rich alluvial silt | 2422 | 3·4 | 6·5 | 1250 | 31·0 | 4·4 | 9·8 | 38·0 | 14·8 | 26·0 | 27·6 | 31·7 | 13·2 | 10.4 |
| Wissington | (*c*) Sandy loam | 2935 | 1·2 | 7·0 | 1300 | 8·2 | 4·6 | 4·0 | 39·6 | 25·4 | 8·5 | 18·8 | 47·3 | 21·5 | 20·8 |
| Peterborough | (*c*) Black fen | 2911 | 14·7 | 6·3 | 1025 | 31·6 | 8·8 | 20·0 | 28·8 | 16·6 | 22·4 | 21·0 | 40·0 | 4·9 | 7·8 |
| Ely | (*c*) Heavy fen | 2861 | 17·1 | 7·5 | 1125 | 31·2 | 8·4 | 18·9 | 32·0 | 11·1 | 26·7 | 29·3 | 32·9 | 2·7 | 2·7 |
| Cockle Park | (*a*) Poor old grassland on boulder clay | 1440 | 2·0 | 5·5 | 300 | 27·3 | 10·0 | 10·3 | 3·3 | 13·3 | 23·3 | 0·0 | 63·4 | 2·7 | 3·3 |
| Chesterfield | (*b*) Old grassland on lower coal measures shale | 2339 | 4·0 | 5·2 | 950 | 25·0 | 12·6 | 25·8 | 5·3 | 14·7 | 35·8 | 3·2 | 46·3 | 1·4 | 2·1 |
| Dartington Hall | (*b*) Old grassland on Devonian shale | 1750 | 6·1 | 5·7 | 1550 | 31·5 | 7·1 | 34·2 | 12·6 | 11·9 | 46·8 | 7·8 | 33·9 | 1·2 | 4·8 |
| Braintree | (*b*) New grassland on calcareous boulder clay | 1730 | 1·7 | 7·4 | 650 | 21·5 | 3·8 | 7·7 | 14·6 | 8·5 | 15·4 | 9·9 | 66·2 | 6·2 | 4·7 |
| Badminton | (*b*) New grassland on Oolite | 2319 | 3·7 | 7·0 | 1250 | 37·5 | 4·4 | 17·6 | 20·0 | 14·0 | 34·0 | 10·4 | 41·6 | 1·3 | 9·6 |
| Bangor | (*a*) Old grassland | 1443 | 3·2 | 5·7 | 2050 | 18·5 | 28·0 | 23·8 | 8·8 | 25·6 | 40·0 | 11·2 | 22·9 | 2·0 | −2·4 |
| Insch | (*a*) From basic igneous rocks | 1438 | 5·1 | 5·8 | 2750 | 10·5 | 18·2 | 28·2 | 34·5 | 11·8 | 30·9 | 40·9 | 16·4 | 0·8 | −6·4 |
| Carbello | (*b*) Arable after grass | 3328 | 6·4 | 5·2 | 1200 | 12·3 | 23·3 | 40·6 | 5·8 | 22·5 | 50·4 | 6·6 | 20·4 | 3·3 | −0·8 |
| **U.S.S.R.:** | | | | | | | | | | | | | | | |
| Lisino | Podzol (A 2) | 1303 | 0·6 | 5·4 | 250 | 23·7 | 22·0 | 18·8 | 10·0 | 26·0 | 22·0 | 6·0 | 46·0 | 1·6 | 4·0 |
| Starajilovo | Grey forest soil (*a*) | 1310 | 1·0 | 5·8 | 450 | 30·7 | 17·8 | 8·9 | 25·6 | 27·8 | 23·3 | 15·6 | 33·3 | 9·3 | 10·0 |
| Veronesh | Cultivated chernozem (K. K. Gedroiz's sample) | 1721 | 5·8 | 6·1 | 900 | 39·7 | 6·1 | 33·3 | 13·9 | 8·9 | 35·6 | 11·9 | 44·4 | 2·6 | 2·8 |
| Isherdeny | Deep chernozem (A 1) | 1314 | 4·2 | 6·3 | 750 | 39·7 | 8·7 | 33·3 | 19·3 | 6·7 | 36·7 | 21·3 | 35·3 | 3·7 | −2·0 |
| Gashun | Deep columnar solonetz (A) | 1327 | 1·6 | 6·5 | 700 | 17·0 | 7·9 | 13·6 | 19·3 | 10·7 | 13·6 | 16·5 | 59·2 | 10·6 | 2·8 |
| Gashun | Chestnut (A 1) | 1330 | 3·4 | 6·8 | 800 | 26·0 | 8·4 | 18·8 | 30·0 | 7·5 | 18·7 | 31·2 | 42·5 | 16·9 | −1·2 |
| Batum | Red soil (A) | 1344 | 3·6 | 4·7 | 1000 | 40·5 | 16·5 | 17·0 | 4·0 | 20·5 | 19·5 | 0·0 | 60·0 | 0·0 | 4·0 |
| **U.S.A.:** | | | | | | | | | | | | | | | |
| Wisconsin | Superior clay | 3334 | 1·6 | 5·1 | 350 | 18·0 | 30·0 | 14·3 | 14·3 | 25·7 | 17·1 | 18·6 | 38·6 | 6·3 | −4·3 |
| Madison, Wis. | Miami silt loam | 3335 | 1·5 | 6·6 | 550 | 14·0 | 13·6 | 10·0 | 19·1 | 23·6 | 10·9 | 9·1 | 56·4 | 10·9 | 10·0 |
| Milwaukee, Wis. | Carrington silt loam | 3333 | 2·7 | 6·1 | 650 | 26·5 | 16·9 | 31·6 | 5·4 | 16·1 | 43·8 | 6·2 | 33·8 | 1·4 | −0·8 |
| Storrs agric. College, Conn. | Pasture plots | 3336 | 3·4 | 5·3 | 1500 | 11·0 | 16·0 | 22·4 | 4·0 | 14·0 | 26·0 | 6·0 | 54·0 | 0·5 | −2·0 |
| **Hawaii:** | | | | | | | | | | | | | | | |
| Kauai | Pineapple soil | 3329 | 4·9 | 4·7 | 2000 | 45·5 | 26·2 | 20·8 | 3·8 | 20·0 | 23·7 | 10·0 | 46·2 | 0·2 | −6·2 |
| Oahu | Pineapple soil | 3331 | 2·2 | 5·2 | 750 | 63·7 | 24·0 | 32·7 | 4·7 | 24·0 | 28·0 | 4·7 | 43·3 | 0·9 | 0·0 |
| Maui | Pineapple soil | 3330 | 3·0 | 4·5 | 2500 | 42·5 | 31·0 | 14·0 | 3·4 | 28·0 | 18·0 | 6·4 | 47·6 | 0·2 | −3·0 |
| **Africa:** | | | | | | | | | | | | | | | |
| Domira Bay, Nyasaland | Grey, neutral | 2789 | 2·2 | 6·0 | 350 | 40·3 | 12·9 | 18·6 | 5·7 | 12·9 | 32·8 | 5·7 | 48·6 | 1·1 | 0·0 |
| | Red, acid | 2797 | 1·3 | 5·6 | 250 | 42·3 | 16·0 | 28·8 | 6·0 | 16·0 | 38·0 | 6·0 | 40·0 | 1·6 | 0·0 |

(*a*) A.E.A. samples. (*b*) From basic slag experiments. (*c*) From sugar-beet experiments.

## APPENDIX II (*a*)

*Analysis of data for thirty-four mineral soils*
*(omitting the two fen soils Nos.* 2911 *and* 2861)

| | | Mean | Standard deviation per soil |
|---|---|---|---|
| *b* | pH value | 5·97 | 0·88 |
| *a* | Carbon % | 2·81 | 1·63 |
| *y* | Clay % | 25·29 | 13·67 |
| *c* | Total P in p.p.m. | 1032 | 214 |
| | *Fractions of soil phosphorus expressed as percentages of total phosphorus* | | |
| | Extractions without sodium acetate: | | |
| *i* | Inorg. alk.-sol. | 15·0 | 8·0 |
| *o* | Org. alk.-sol. | 19·4 | 10·0 |
| *e* | Inorg. acid-sol. | 14·7 | 11·1 |
| | Extractions with sodium acetate: | | |
| *t* | Inorg. alk.-sol. | 18·9 | 7·5 |
| *k* | Org. alk.-sol. | 27·0 | 10·2 |
| *x* | Estimated acid-sol. (= *e* − *v*) | 10·9 | 9·2 |
| | Other fractions: | | |
| *m* | Insoluble (= 100 − *i* − *e* − *k*) | 43·2 | 12·3 |
| *g* | Truog acid-sol. | 5·3 | 5·5 |
| *v* | Increase in inorg. alk.-sol. through use of NaAc (= *t* − *i*) | 3·8 | 7·3 |

## APPENDIX II (*b*)

*Regression coefficients of fractions as percentages of total phosphorus on* pH, *carbon, and clay*

| | | On pH *b* | On pH *b* | On carbon *a* | On pH *b* | On carbon *a* | On clay *y* | Standard errors of coefficients: pH *b* | pH *b* | Carbon *a* | pH *b* | Carbon *a* | Clay *y* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Extractions without NaAc:** | | | | | | | | | | | | |
| *i* | Inorg. alk.-sol. | −6·99‡ | −7·51‡ | −1·23* | −7·92‡ | −1·07 | −0·088 | 1·02 | 0·98 | 0·53 | 1·02 | 0·54 | 0·066 |
| *o* | Org. alk.-sol. | −4·83† | −3·15* | +3·96‡ | −2·86 | +3·86‡ | −0·062 | 1·83 | 1·37 | 0·74 | 1·45 | 0·76 | 0·094 |
| *e* | Inorg. acid-sol. | +7·93‡ | +8·33‡ | +0·95 | +7·78‡ | +1·15 | −0·118 | 1·74 | 1·79 | 0·97 | 1·88 | 0·99 | 0·122 |
| | **Extractions with NaAc:** | | | | | | | | | | | | |
| *t* | Inorg. alk.-sol. | −2·40 | −3·14* | −1·74* | −3·72† | −1·53 | −0·125 | 1·45 | 1·40 | 0·75 | 1·45 | 0·76 | 0·094 |
| *k* | Org. alk.-sol. | −2·92 | −1·30 | +3·85‡ | −1·14 | +3·70‡ | +0·034 | 1·99 | 1·64 | 0·88 | 1·74 | 0·92 | 0·113 |
| *x* | Estimated acid-sol. | +3·34 | +3·96* | +1·46 | +3·58 | +1·60 | −0·081 | 1·75 | 1·76 | 0·95 | 1·87 | 0·98 | 0·121 |
| | **Other fractions:** | | | | | | | | | | | | |
| *m* | Insoluble | +1·99 | +0·48 | −3·58‡ | +1·28 | −3·87‡ | +0·172 | 2·45 | 2·26 | 1·22 | 2·36 | 1·24 | 0·153 |
| *g* | Truog | +4·36‡ | +4·08‡ | −0·68 | +3·72‡ | −0·53 | −0·079 | 0·81 | 0·81 | 0·44 | 0·84 | 0·44 | 0·054 |
| *v* | Increase in alk.-sol. through NaAc | +4·59‡ | +4·37‡ | −0·61 | +4·20‡ | −0·45 | −0·037 | 1·23 | 1·27 | 0·69 | 0·93 | 0·49 | 0·060 |

* $P < 0·05 > 0·02$. † $P < 0·02 > 0·01$. ‡ $P < 0·01$.

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# THE NATURE OF THE COMPETITION BETWEEN CEREAL CROPS AND ANNUAL WEEDS

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(With Five Text-figures)

## Introduction

Although it has long been realized that the presence of annual weeds depresses the yield of cereal crops, yet no detailed study has been made of the principal factors governing such competition. During the last thirty years, numerous papers have been published dealing with methods of weed control. With the exception of Korsmo (1932), the authors have been content to demonstrate the effectiveness of the various methods without measuring the increase in crop yield obtained as a result of weed suppression. Korsmo in Norway carried out a large number of simple trials, in which he recorded the increases in crop yield following the eradication of weeds. He found that the most effective treatments, such as spraying with sulphuric or nitric acid solutions, increased the yield of cereals on an average by some 25 %. Korsmo concluded on somewhat indirect evidence that competition between the weeds and the crop was in the main for water and soil nutrients. He did not, however, determine which of the soil nutrients was of major importance, or whether the intensity or the nature of the competition varied both with the crop and the weed species.

In two earlier papers (Blackman, 1934; Blackman & Templeman, 1936), dealing with the eradication of weeds from cereals by means of sulphuric acid solutions, the increases in yield due to weed suppression were very variable. The data collected over four years from 1932 to 1935 indicated that this variability was in part due to seasonal differences. The presence of weeds had a greater depressing effect on the yield of the cereal in a wet spring. Other experiments indicated that competition between the weeds and the crop was for soil nutrients. In one or two experiments, however, the complete suppression of a larger proportion of the cereal plants in the weedy crop indicated that besides soil nutrients, there were in addition other factors involved, probably competition for light or water.

### Experimental results

#### *The relationship between the density of the weed species and the intensity of the competition with the cereal*

The earlier investigations (Blackman, 1934; Blackman & Templeman, 1936), demonstrated that the order of the increases in yield obtained following upon the eradication of the weeds was related to the weed species. In Fig. 1 the results for all the experiments in the four years are summarized. Here the percentage increases in yield obtained as a result of weed suppression have been plotted against the density of the weed on the unsprayed plots. It is clear, in spite of the large fluctuations,

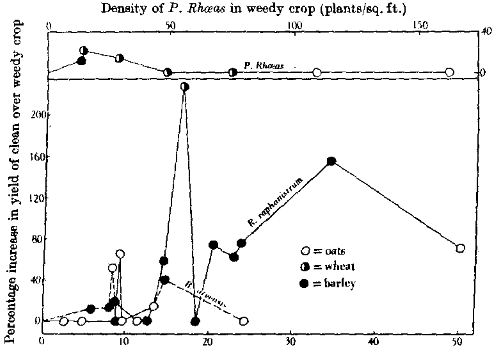


Fig. 1. The percentage increases in crop yield following upon the eradication of either *B. arvensis*, *R. raphanistrum* or *P. Rhœas* from cereals. Data obtained from various experiments during 1932–5.

that the eradication of *Raphanus raphanistrum* leads to the greatest increases in yield, while the control of *Papaver Rhœas* may have little effect. The data in Fig. 1 show that the intensity of competition is dependent upon the species of weed but no accurate comparison of their relative competitive powers can be made as the experiments with the different weed species were seldom carried out in the same field, on the same crop, and in the same season.

In order to obtain reliable information on the competitive powers of

different weeds, experiments were laid down at Jealott's Hill in both 1934 and 1935. They consisted of large random blocks in triplicate, a set of control plots being included for each weed species. Seed of *Brassica arvensis*, *P. Rhœas* and *R. raphanistrum* was sown at varying rates immediately after the cereal crop had been drilled. Seed of *P. Rhœas* and *B. arvensis*, obtained direct from farmers, was easily cleaned, and the samples showed a satisfactory germination. With *R. raphanistrum* difficulty was experienced. In the first place, it was not possible to separate the lomenta from the grain of barley and oats since differences in size and shape were too small. In the second place, germination tests showed that no seedlings appeared above the soil for some weeks after sowing, i.e. until the pericarp had rotted. In the field this rotting would normally take place between the shedding of the seed and the next spring. In order to ensure rapid germination in the field experiments, an attempt was made to remove the pericarp without damaging the seed. The lomenta were first dried to make the pericarp brittle, and passed through an oat crusher suitably adjusted. The material was screened to remove any seed liberated, and the process repeated. This method had the advantage of destroying most of the barley and oat grain, but had also the disadvantage of damaging much of the seed of *R. raphanistrum*. In fact, from 5 cwt. of material only some 30 lb. of seed was obtained in each year.

In 1934 experiments were carried out on both spring oats and barley. In the oat experiment all three weed species were included. There were six rates of seeding for each species. The quantity of seed sown was doubled for each successive rate, i.e. the highest rate was thirty-two times the lowest rate. In the barley experiment *R. raphanistrum* had perforce to be omitted owing to a lack of seed. Instead a smaller separate experiment with only four rates of seeding was laid down on an adjacent site.

In the statistical analysis of the data the original intention was to use the method of analysis of variance, since it was hoped that for each rate of seeding the density of the weed species would be similar for each replicate. But the depredations of birds, particularly at the higher rates of seeding, produced large differences in density between replicates. Regressions of the effect of increasing weed density on the yield have therefore been fitted to the data. Estimates of density were obtained by counting the number of seedlings in 20–30 quadrats (0·25 or 1·0 sq. ft.) chosen at random on each plot (0·0125 acre).

The results of the 1934 experiments, together with the fitted regression

equations, are shown in Figs. 2 and 3. In Fig. 2 the regression of density on the yield of oats is significant only in the case of *R. raphanistrum*. There is no evidence that the presence of *P. Rhœas* depressed the crop yield, even though the mean density was much higher than that of either *R. raphanistrum* or *B. arvensis*, namely 47·6 plants per square foot, as against 12·5 or 25·1 plants. In the two barley experiments, *vide* Fig. 3, no depression in yield has resulted from the presence of any of the weed species, since none of the regressions are significant. But it should be observed that the average density for each species is considerably

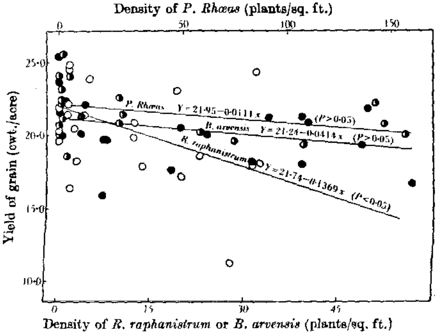


*Fig.* 2. The relationship, expressed as a straight line regression, between the yield of spring oats and the density of the weed growing in the crop. Data for *R. raphanistrum* shown as white circles, for *B. arvensis* as black circles and for *P. Rhœas* as half-black circles. (Jealott's Hill, 1934.)

smaller than in the oat experiment, i.e. 7·0, 10·9, and 36·5 plants per square foot for *R. raphanistrum*, *B. arvensis* and *P. Rhœas* respectively.

In 1935, a similar experiment to the 1934 barley trial was laid down, but the rates of seeding for both *B. arvensis* and *P. Rhœas* were increased. The results of this experiment are given in Fig. 4. Competition between *B. arvensis* and the barley has led to a significant reduction in yield, but the regression for *P. Rhœas* is not significant even though the average density was 205 plants per square foot.

Both 1934 and 1935 were unsuitable seasons for carrying out field experiments with low errors. In 1934 the large variation in yield between replicates was in part due to the drought. Differences in the subsoil were

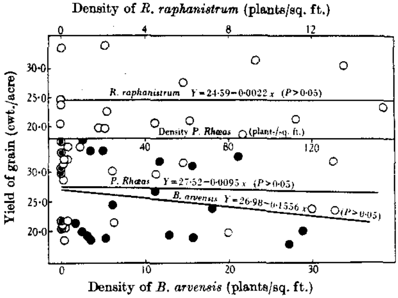


Fig. 3. The relationship, expressed as a straight line regression, between the yield of spring barley and the density of the weed growing in the crop. In the lower half of the figure, the data for *B. arvensis* are shown as black circles, and those for *P. Rhœas* as white circles. (Jealott's Hill, 1934.)

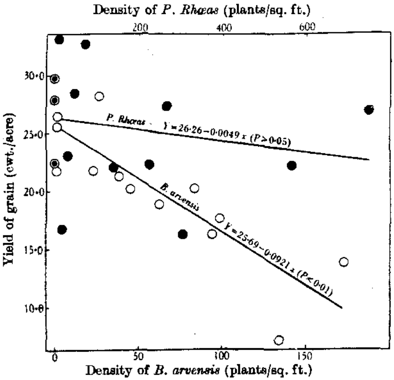


Fig. 4. The relationship, expressed as a straight line regression, between the yield of spring barley and the density of the weed growing in the crop. Data for *B. arvensis* shown as black circles, those for *P. Rhœas* as white circles. (Jealott's Hill, 1935.)

reflected in the crop. The effects of a low soil moisture content showed up as irregular small patches where gravel pockets occurred in the clay. In 1935, the heavy rainfall in April led to waterlogging in the field where the experiments were carried out. In consequence, some of the seed rotted in the ground and germination of the barley was irregular. The large series of experiments on oats which had been laid out in the lower half of the field were more severely waterlogged and had to be abandoned. In addition, although the establishment of the three weed species was not seriously affected by these conditions, the seedlings of *R. raphanistrum* were almost completely destroyed by turnip flea beetles (*Phyllotreta undulata* and *Plectroscelis concinna*).

*The nature of the competition between the weed and the cereal. The influence of nitrogen supply on the development and yield of the cereal in the presence and absence of weeds*

The experiments designed to give information on the nature of the competition between annual weeds and cereals have been largely carried out on spring sown crops. In such crops, weeds are both more prevalent and more troublesome than in autumn-sown cereals. Most attention has been given to studying the competition of *B. arvensis* and *R. raphanistrum* for these weeds are the two commonest annual species in arable land.

On *a priori* grounds it was thought that competition for nutrients would be an important factor since many workers have demonstrated that in cereals salt absorption is most active in the early stages of development, i.e. at a time when weeds are also growing rapidly. In the case of barley, Gregory and his co-workers (1937) have shown that nitrogen, phosphorus and potassium are taken up most actively during the vegetative phase, while Brenchley (1929) has proved that all the necessary phosphorus is absorbed in the early stages. Wagner (1932), Garola (1934), Blank & Giesecke (1934) and Stahl & Shive (1933) report similar observations on oats. Numerous manurial experiments carried out in England have shown that under normal rotational farming considerable increases in the yield of spring cereals have followed upon nitrogenous manuring, but that the responses to phosphorus and potassium have been small. Therefore it was decided to investigate in the first place the importance of nitrogen supply.

In all the 1934 experiments and one or two of the 1935 experiments, nitrogen, as nitrochalk, was applied at a single rate (23·25 lb. nitrogen per acre). In the majority of the 1935 trials two rates of application were made (23·25 and 46·5 lb. nitrogen per acre). The experiments

consisted of replicated random blocks, the plot size varying from 0·01 to 0·0125 acre. In the Jealott's Hill experiments the weedy plots were obtained by sowing seed in the same way as previously described (see p. 249). In the other experiments, fields naturally infested with the appropriate weeds were chosen. In order to obtain "clean" experimental plots, the weeds were removed when still in the young seedling stage. In only one of the experiments away from Jealott's Hill was it found possible to remove the weeds even partially by hand on account of the time taken and labour involved. In all the other experiments, the weeds were eradicated by spraying with sulphuric acid solutions, or in the case of the experiment on *Chrysanthemum segetum* with sulphuric acid and a wetting agent. Subsequent to spraying the density of the weed on the sprayed and unsprayed plots was determined by counting the number of individuals found in some 15–30 quadrats chosen at random on each plot. The degree of control obtained with concentrations of 9·2–13·8 % acid have been reported previously (Blackman, 1934; Blackman and Templeman, 1936); it ranged from 91·8 to 99·7 % for *B. arvensis* and *R. raphanistrum* and 84 % in the case of *C. segetum*.

The results of the experiments in which nitrogen as nitrochalk was added at the single rate to the crop in the presence and absence of weeds are given in Table I. At North Petherton and Broadclyst it is seen that the yield of weed-free barley or barley containing either *B. arvensis* or *C. segetum* has been significantly increased by the application of nitrochalk. By the addition of nitrogen the yield of the weedy crop has been raised to the same level as that of the clean crop. On the other hand, at Bury St Edmunds, top dressing with nitrogen has only increased the yield in the clean crop, while at Jealott's Hill, although the presence of *B. arvensis* has not depressed the yield of oats, there is some indication that the addition of nitrogen has increased the difference in yield between the clean and weedy crop. Finally, applying nitrogen to barley in the presence and absence of *R. raphanistrum* (Holyport and Milverton) has increased neither the yield of the clean nor of the weedy crop, although the depressions in yield due to *R. raphanistrum* are much larger than in the experiments on *B. arvensis* or *C. segetum*.

In order to interpret with greater clarity the changes in crop yield brought about by nitrogenous manuring in the presence and absence of weeds, a study was made of the cereal's development under such conditions in six of the 1935 experiments. Estimates of tiller and shoot production were obtained by counting 5–10 individual yard rows chosen at random on each plot and estimates of height were made by measuring

to the tip of the longest leaf 10–20 plants per plot. The number of fertile grains per ear was determined by observing 50–60 ears per plot, while the thousand grain weight was obtained for each plot from a random sample of the *threshed* grain. Such observations were carried out on six of the 1935 experiments and involved some 20,000 counts.

Table I. *The influence of additional nitrogen on crop yield in the presence and absence of weeds*

| Centre ... | North Petherton | | Broadclyst | | Bury St Edmunds | |
|---|---|---|---|---|---|---|
| Year ... | 1935 | | 1935 | | 1934 | |
| *Crop* ... | Barley | | Barley | | Barley | |
| Weed species ... | *B. arvensis* | | *C. segetum* | | *B. arvensis* | |
| | Weed density plants/sq. ft. | Crop yield cwt./acre | Weed density plants/sq. ft. | Crop yield cwt./acre | Weed density plants/sq. ft. | Crop yield cwt./acre |
| Weeds present: | | | | | | |
| Control | 5·5 | 20·0 | 77·4 | 14·80 | 8·2 | 19·08 |
| 23·25 lb. N/acre | 6·5 | 23·57 | 87·3 | 17·10 | 8·9 | 18·86 |
| Weeds absent: | | | | | | |
| Control | 0·1 | 22·57 | 10·9 | 17·17 | 0·6 | 21·48 |
| 23·25 lb. N/acre | 0·1 | 27·52 | 15·6 | 21·08 | 0·8 | 25·73 |
| Sig. diff. ($P=0·05$) | — | 2·35 | — | 2·20 | — | 2·18 |

| Centre ... | Jealott's Hill | | Holyport | | Milverton | |
|---|---|---|---|---|---|---|
| Year ... | 1934 | | 1934 | | 1934 | |
| Crop ... | Oats | | Barley | | Barley | |
| Weed species ... | *B. arvensis* | | *R. raphanistrum* | | *R. raphanistrum* | |
| | Weed density plants/sq. ft. | Crop yield cwt./acre | Weed density plants/sq. ft. | Crop yield cwt./acre | Weed density plants/sq. ft. | Crop yield cwt./acre |
| Weeds present: | | | | | | |
| Control | 12·8 | 21·39 | 14·7 | 8·54 | 23·2 | 13·00 |
| 23·25 lb. N/acre | 11·7 | 20·35 | 14·4 | 8·71 | 24·6 | 15·63 |
| Weeds absent: | | | | | | |
| Control | 0 | 20·85 | 0·2 | 13·45 | 1·3 | 20·98 |
| 23·25 lb. N/acre | 0 | 22·57 | 0·4 | 14·45 | 0·7 | 23·05 |
| Sig. diff. ($P=0·05$) | — | 5·09 | — | 3·67 | — | 6·90 |

*The influence of* Brassica arvensis *on the growth of barley.* The effect of *B. arvensis* and nitrogenous manuring on the growth of barley was observed in two of the 1935 experiments at Medmenham and Ilminster. As it has already been pointed out (*vide* p. 252), nitrogen was applied at the rate of 23·25 and 46·5 lb./acre to the weedy and clean crop.

The data in Table II show that at the lowest nitrogen level competition between *B. arvensis* and the barley has resulted primarily in a decreased tiller and fertile shoot production and secondarily, to a lesser extent, has reduced the number of fertile grains per ear.

Table II. *The growth of spring barley as affected by nitrogen supply and by competition with* Brassica arvensis. *Medmenham* 1935

Variety: Spratt-Archer. Soil: Clay with flints over chalk. Cropping: Oats in 1934. Manuring: 2 cwt. superphosphate, 1 cwt. kainit, 1 cwt. ammonium sulphate per acre at seeding in both 1934 and 1935.

| Treatments | | Tillers per yard row | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Nitrogen supply lb./acre | Degree of weed infestation plants/sq. ft. | 27 May | 14 June | 7 July | Fertile shoots per yard row | Grains per ear | 1000 grain weight g. | Yield of grain cwt./acre |
| Control | 14·4 | 52·5 | 57·6 | 49·7 | 23·2 | 20·6 | 36·2 | 8·42 |
| 23·25 | 12·5 | 58·9 | 71·3 | 56·4 | 36·4 | 22·5 | 35·7 | 13·00 |
| 46·5 | 12·3 | 63·5 | 73·0 | 56·7 | 41·1 | 22·6 | 35·4 | 13·97 |
| Control | 1·7 | 59·3 | 99·2 | 77·7 | 37·0 | 22·8 | 37·3 | 11·83 |
| 23·25 | 1·5 | 61·5 | 107·1 | 86·7 | 43·5 | 24·5 | 38·1 | 15·66 |
| 46·5 | 2·8 | 74·4 | 117·9 | 91·1 | 44·0 | 24·2 | 36·2 | 15·28 |
| Sig. diff. ($P = 0{\cdot}05$) | | 17·3 | 14·2 | 12·6 | 8·4 | 1·7 | 1·3 | 5·05 |

The addition of nitrogen to the weedy crop has significantly increased the number of tillers, shoots and grains per ear. In the clean crop nitrogen has had a similar effect, although to a smaller degree than in the weedy crop.

The large error attached to the yield data can be attributed to the depredations of rabbits during the last few weeks prior to harvest when a considerable number of the fertile shoots were bitten through. Shoots damaged in this way have been included in the count. A more accurate assessment of the yield differences can therefore be obtained from a consideration of shoot, number of grains per ear and grain weight data. On this basis, the presence of *B. arvensis* has significantly depressed the yield, while nitrogen applied at the rate of 23·25 lb./acre has raised the level of yield to that of the clean crop.

Table III. *The growth of spring barley as affected by nitrogen supply and by competition with* Brassica arvensis. *Ilminster* 1935

Variety: Spratt-Archer. Soil: Sandstone loam. Cropping: Mangolds in 1934. Manuring: Farmyard manure in 1934, 2 cwt./acre "complete" fertilizer at seeding in 1935.

| Treatments | | | | | | |
|---|---|---|---|---|---|---|
| Nitrogen supply lb./acre | Degree of weed infestation plants/sq. ft. | Tillers per yard row 20 June | Fertile shoots per yard row | Grains per ear | 1000 grain weight g. | Yield of grain cwt./acre |
| Control | 8·3 | 62·0 | 47·3 | 21·5 | 32·4 | 17·03 |
| 23·25 | 11·4 | 69·0 | 57·3 | 22·3 | 33·1 | 18·73 |
| 46·5 | 11·6 | 79·9 | 69·7 | 22·2 | 33·8 | 22·92 |
| Control | 1·7 | 71·5 | 54·3 | 22·5 | 32·7 | 20·20 |
| 23·25 | 2·0 | 84·9 | 74·6 | 24·1 | 33·5 | 24·89 |
| 46·5 | 2·3 | 104·3 | 90·7 | 25·4 | 33·[illegible] | 29·33 |
| Sig. diff. ($P = 0{\cdot}05$) | | 6·4 | 8·2 | 1·8 | 1·6 | 3·64 |

It is seen from the data in Table III that competition between the barley and *B. arvensis* has depressed yield through decreasing tiller and shoot production. There has been no marked depression in ear size or grain weight. The addition of nitrogen either to the clean or the weedy crop has brought about an increase in tiller and shoot production, and also in the case of the weed free crop an increase in ear size. The response of the clean crop to additional nitrogen is greater than in the weedy crop; nevertheless increasing the nitrogen supply in the weedy crop has raised the level of yield to that of the clean crop receiving no nitrogen.

*The influence of* Raphanus raphanistrum *on the growth of barley.* Similar observations to those described in the previous section were carried out on barley in two field trials. At Dorchester (*vide* Table IV) competition by *R. raphanistrum* has led principally to a reduction in the number of fertile shoots and in a lesser degree to a decreased tiller production. A diminution in ear size has also been brought about. In the clean crop, nitrogen manuring has increased the tiller and fertile shoot production. At the higher rate of manuring the ear size has heen increased and the grain weight depressed. In the presence of *R. raphanistrum*, the addition of nitrogen has not increased the tiller production but has nevertheless increased the number of fertile shoots. As in the clean crop, nitrogen has increased ear size. Additional nitrogen has increased the yield of the weedy crop more than that of the clean barley. In consequence, the weedy crop receiving the highest rate of application approached the yield level of the unmanured clean crop.

Table IV. *The growth of spring barley as affected by nitrogen supply and by competition with* Raphanus raphanistrum. *Dorchester* 1935

Variety: Spratt-Archer. Soil: Alluvial loam. Cropping: Wheat in 1934. Manuring: 2 cwt. superphosphate, 1 cwt. kainit, 1 cwt. ammonium sulphate per acre at seeding in both 1934 and 1935.

| Treatments | | Tillers per yard row | | | Fertile shoots per yard row | Grains per ear | 1000 grain weight g. | Yield of grain cwt./acre |
|---|---|---|---|---|---|---|---|---|
| Nitrogen supply lb./acre | Degree of weed infestation plants/sq. ft. | 20 May | 6 June | 28 July | | | | |
| Control | 20·7 | 123·4 | 94·6 | 91·8 | 55·8 | 17·1 | 31·4 | 13·00 |
| 23·25 | 20·6 | 116·1 | 111·1 | 77·3 | 62·2 | 19·9 | 33·0 | 16·50 |
| 46·5 | 18·9 | 124·6 | 119·3 | 81·1 | 73·0 | 19·6 | 30·8 | 18·95 |
| Control | 0·9 | 117·1 | 123·7 | 96·6 | 82·4 | 22·1 | 34·5 | 22·57 |
| 23·25 | 1·5 | 114·5 | 149·8 | 109·8 | 91·5 | 23·8 | 33·6 | 26·70 |
| 46·5 | 0·6 | 116·7 | 160·2 | 106·5 | 94·7 | 24·1 | 31·2 | 26·05 |
| Sig. diff. ($P=0·05$) | | 14·8 | 8·5 | 6·6 | 8·5 | 1·8 | 2·1 | 4·36 |

In the Milverton experiment (Table V), the presence of *R. raphanistrum* in the barley has again depressed fertile shoot production to a greater extent than tiller production.

Both the ear size and grain weight have also been reduced by weed competition. The addition of nitrogen to either the clean or weedy crop has primarily increased the number of tillers and has not, particularly in the clean crop, proportionally increased the number of shoots. Nitrogen at the higher rate of application has increased the ear size in the clean, but not in the weedy crop. Contrary to the findings of the three previous barley experiments, additional nitrogen has not increased the yield of the clean crop or even that of the weedy crop, in spite of the large depression brought about by *R. raphanistrum.*

Table V. *The growth of spring barley as affected by nitrogen supply and by competition with* Raphanus raphanistrum. *Milverton* 1935

Variety: Spratt-Archer. Soil: Sandstone loam. Cropping: Root crop failed in 1934. Manuring: 15·5 lb. N, 62 lb. $P_2O_5$, 22 lb. $K_2O$/acre in 1934, no fertilizers in 1935.

| Treatments: Nitrogen supply lb./acre | Treatments: Degree of weed infestation plants/sq. ft. | Tillers per yard row: 29 May | Tillers per yard row: 20 June | Fertile shoots per yard row | Grains per ear | 1000 grain weight g. | Yield of grain cwt./acre |
|---|---|---|---|---|---|---|---|
| Control | 34·8 | 72·9 | 53·6 | 38·9 | 17·0 | 32·0 | 11·67 |
| 23·25 | 35·3 | 77·9 | 60·0 | 41·5 | 17·1 | 32·0 | 11·94 |
| 46·5 | 33·9 | 86·8 | 64·6 | 48·5 | 18·4 | 32·2 | 14·33 |
| Control | 2·9 | 100·9 | 102·2 | 77·9 | 24·5 | 37·6 | 29·71 |
| 23·25 | 2·4 | 124·6 | 120·3 | 86·5 | 26·1 | 36·7 | 32·39 |
| 46·5 | 2·1 | 139·0 | 135·4 | 86·9 | 26·5 | 36·5 | 30·04 |
| Sig. diff. ($P$ = 0·05) | | 12·1 | 15·6 | 5·2 | 1·8 | 2·5 | 3·35 |

*The influence of* Raphanus raphanistrum *on the growth of oats.* The growth of oats at three different nitrogen levels in the presence and absence of *R. raphanistrum* was studied in two experiments at Hascombe and Holyport.

Table VI. *The growth of oats as affected by nitrogen supply and by competition with* Raphanus raphanistrum. *Hascombe* 1935

Variety: Marvellous. Soil: Greensand. Cropping: Kale in 1934. Manuring: 18 tons/acre of farmyard manure in 1934, no fertilizers in 1935.

| Treatments: Nitrogen supply lb./acre | Treatments: Degree of weed infestation plants/sq. ft. | Tillers per yard row: 13 June | Tillers per yard row: 3 July | Fertile shoots per yard row | Grains per panicle | 1000 grain weight g. | Yield of grain cwt./acre |
|---|---|---|---|---|---|---|---|
| Control | 8·6 | 36·0 | 28·4 | 24·4 | 28·2 | 36·9 | 12·43 |
| 23·25 | 10·6 | 43·0 | 34·9 | 32·8 | 38·9 | 35·9 | 19·80 |
| 46·5 | 9·9 | 48·6 | 37·0 | 37·2 | 45·2 | 35·6 | 22·90 |
| Control | 1·0 | 51·4 | 48·8 | 37·6 | 42·3 | 35·6 | 20·51 |
| 23·25 | 0·9 | 60·9 | 47·7 | 37·3 | 53·8 | [illegible]4·6 | 24·46 |
| 46·5 | 0·5 | 88·4 | 57·6 | 48·5 | 53·9 | 32·6 | 28·91 |
| Sig. diff. ($P$ = 0·05) | | 12·4 | 8·8 | 10·2 | 8·3 | 1·3 | 2·50 |

From the data in Table VI, it is seen that as in previous experiments, the competition between the weed and the cereal has led to a reduction in both the number of tillers and fertile shoots. In this experiment, the number of grains per panicle and, to a smaller extent, the grain weight, have been reduced. Nitrogen applied at the lower rate has primarily increased the panicle size both in the clean and the weedy crop. At the higher rate, there has been an increased tiller and shoot production but a diminution in grain weight. Nitrogenous manuring has raised the yield of the weedy crop to the same level as that of the clean crop receiving no nitrogen.

At Holyport the experiment was of a more elaborate nature than those previously described. In addition to the usual six treatments (three levels of nitrogen supply in both the clean and weedy crop), further treatments were included in order to determine the period of greatest competition. *R. raphanistrum* was removed at different stages of development. The first set of plots were hand weeded when *R. raphanistrum* was in the seedling stage, i.e. at the time when spraying with sulphuric acid was carried out. The second set were hoed 16 days later, while in the third set the weed was hand pulled a fortnight later when it was in full flower.

Table VII. *The growth of oats as affected by nitrogen supply and by competition with* Raphanus raphanistrum. *Holyport* 1935

Variety: Victory. Soil: Brick earth. Cropping: Clover ley in 1934. Manuring: No fertilizers in 1934, 23·5 lb. N, 108 lb. $P_2O_5$, and 20 lb. $K_2O$/acre at seeding in 1935.

| Treatments | Density *R. raphanistrum* plants/sq. ft. | Tillers per yard row 3 May | 16 May | 31 May | 1 July | Fertile shoots per yard row | Grains per panicle | 1000 grain weight g. | Yield of grain cwt./acre |
|---|---|---|---|---|---|---|---|---|---|
| Control (*a*) | 54·6 | 67·1 | 59·2 | 62·0 | 61·2 | 50·2 | 24·5 | 30·6 | 13·35 |
| 23·25 lb. N/acre | 46·5 | 59·8 | 59·9 | 60·1 | 61·9 | 55·4 | 28·1 | 29·5 | 16·25 |
| 46·5 lb. N/acre | 42·5 | 66·0 | 54·9 | 74·0 | 57·5 | 53·9 | 32·8 | 29·7 | 15·98 |
| Weeds removed by $H_2SO_4$: | | | | | | | | | |
| Control (*b*) | 0·6 | 65·6 | 61·4 | 83·5 | 75·8 | 60·1 | 46·2 | 28·4 | 22·50 |
| 23·25 lb. N/acre | 1·5 | 69·5 | 67·7 | 95·1 | 82·4 | 70·5 | 52·7 | 27·4 | 25·33 |
| 46·5 lb. N/acre | 0·6 | 64·1 | 67·0 | 99·6 | 82·3 | 65·6 | 51·8 | 28·5 | 25·94 |
| Weeds removed by hand: | | | | | | | | | |
| Seedling stage | 0·2 | 71·6 | 62·2 | 87·7 | 76·2 | 62·8 | 44·9 | 31·5 | 21·56 |
| Pre-flowering stage | 50·8 | 65·0 | 57·6 | 63·8 | 61·0 | 54·1 | 24·6 | 31·4 | 12·37 |
| Flowering stage | 45·6 | 63·7 | 64·2 | 65·6 | 69·2 | 56·6 | 21·4 | 31·1 | 11·61 |
| Sig. diff. ($P = 0·05$): | | | | | | | | | |
| (1) Between treatments | — | 9·4 | 9·4 | 6·6 | 12·7 | 8·6 | 4·9 | 3·0 | 1·91 |
| (2) Between treatments and control (*a*) | — | 8·1 | 8·2 | 5·8 | 11·0 | 7·5 | 4·3 | 2·6 | 1·6[illegible] |

From the data in Table VII it is seen that the presence of *R. raphanistrum* in the oat crop has primarily depressed the number of grains per

panicle. The number of tillers and fertile shoots have also been reduced, but only to a smaller extent. In the weedy crop, nitrogenous manuring at the higher rate has significantly increased both panicle size and tiller production, but it has not increased the number of fertile shoots. In the clean crop, nitrogen added at the lower rate has significantly increased not only the number of tillers and fertile shoots, but also the panicle size. The addition of further nitrogen produced no effect. As in the barley experiment at Milverton, nitrogenous manuring has not raised the yield level of the weedy crop to that of the clean crop. In spite of the high weed density, there has only been significant response to nitrogen applied at the lower rate.

It has already been pointed out that on the evidence of previous investigations (Blackman, 1934; Blackman & Templeman, 1936) spraying with sulphuric acid does not appreciably damage the cereal crop. In this experiment sulphuric acid spraying has had no different effect on either the development or yield than hand-weeding carried out at the same time, i.e. wheu *R. raphanistrum* was in the seedling stage. From the growth data in relation to the time when *R. raphanistrum* was removed from the crop, it can be concluded that the intensity of competition between the weed and the crop has already passed the maximum before *R. raphanistrum* is in full flower.

*The influence of nitrogen supply on crop height and weed height*

In addition to the developmental data already cited, measurements of the height of both the crop and the weed were also made. With the exception of the first set of observations at Holyport, these observations were carried out when the weed had reached the flowering stage. Owing to the lax nature of the cereal leaves, an accurate measure of height presents some difficulty. In this investigation the height from ground level to the tip of the tallest leaf was determined. This estimate exaggerates the true height by some 10–20% depending on the stage of development. These measurements are summarized in Table VIII.

The data in Table VIII show that with the exception of the experiment at Milverton, the height of the crop does not vary appreciably in the presence or absence of weeds. The addition of nitrogen to both the weedy and weed-free crop has increased the crop height. When the weeds are in the seedling stage, the crop tends to exceed in height the weed, *vide* the first count at Holyport. By the time either *R. raphanistrum* or *B. arvensis* is in flower, the crop and the weed are of equal height especially at the lower nitrogen levels.

Table VIII. *Influence of nitrogen supply on crop height and weed height. (In order to distinguish the clean crop from the crop competing with the weed, the weedy crop has been bracketed with the weed)*

| Centre and date of sampling | Crop and weed species | Height (in.) lb. N/acre 0 | 23·25 | 46·5 |
|---|---|---|---|---|
| Hascombe, 14. vi. 35 | Oats | 19·4 | 23·8 | 25·5 |
| | {Oats | 18·6 | 23·9 | 26·0 |
| | {*R. raphanistrum* | 19·8 | 22·2 | 22·0 |
| Holyport, 3. v. 35 | Oats | 11·1 | 11·1 | 11·4 |
| | {Oats | 11·6 | 12·4 | 13·3 |
| | {*R. raphanistrum* | 4·9 | 5·2 | 5·8 |
| Holyport, 31. v. 35 | Oats | 15·8 | 17·9 | 18·7 |
| | {Oats | 14·5 | 17·7 | 19·8 |
| | {*R. raphanistrum* | 13·6 | 14·5 | 15·1 |
| Dorchester, 6. vi. 35 | Barley | 16·8 | 19·3 | 21·1 |
| | {Barley | 15·8 | 18·4 | 20·0 |
| | {*R. raphanistrum* | 11·6 | 14·4 | 15·9 |
| Milverton, 20. vi. 35 | Barley | 24·2 | 27·0 | 27·9 |
| | {Barley | 19·6 | 21·3 | 26·4 |
| | {*R. raphanistrum* | 18·6 | 20·3 | 20·0 |
| Ilminster, 21. vi. 35 | Barley | 24·8 | 30·2 | 33·6 |
| | {Barley | 25·8 | 29·1 | 29·2 |
| | {*B. arvensis* | 19·3 | 20·8 | 23·3 |
| Medmenham, 14. vi. 35 | Barley | 17·0 | 19·1 | 19·8 |
| | {Barley | 15·7 | 17·8 | 19·7 |
| | {*B. arvensis* | 13·6 | 16·8 | 17·3 |

### *Influence of nitrogen supply and weed competition on the mineral content of the cereal*

*Changes in the nitrogen content.* On the basis of other investigations (see p. 252), it seemed likely that the presence of weeds might depress the rate at which nutrients were removed from the soil by cereals in the early stages of growth. In order to obtain further information on this point, in six of the experiments a number of cereal plants selected at random were removed from each plot and the nitrogen contents determined. The analyses were carried out on the shoot after all roots had been first removed. The results of these analyses are given in Table IX.

The changes in nitrogen content brought about either by weeds or nitrogenous manuring show very similar trends in each of the six experiments. The presence of either *B. arvensis* or *R. raphanistrum* has led to a marked reduction in the nitrogen content. The addition of nitrogen to the weedy crop has in every case raised the nitrogen level approximately to that of the clean crop receiving no nitrogen. Similarly the application of nitrogen to the clean crop has also considerably increased the nitrogen content.

Table IX. *The effect of nitrogenous manuring on the nitrogen content of the cereal in the presence and absence of weeds*

| Centre ... | Ilminster | Medmenham | Dorchester | Milverton | Hascombe | Holyport |
|---|---|---|---|---|---|---|
| Crop ... | Barley | Barley | Barley | Barley | Oats | Oats |
| Weed species ... | *B. arvensis* | *B. arvensis* | *R. raphanistrum* | *R. raphanistrum* | *R. raphanistrum* | *R. raphanistrum* |
| Date of sampling | 20. vi. 35 | 14. vi. 35 | 12. vi. 35 | 29. v. 35 | 14. vi. 35 | 4. vi. 35 |
| | Nitrogen content (percentage of dry matter) | | | | | |
| Weeds present: | | | | | | |
| Control | 1·54 | 1·67 | 1·14 | 1·68 | 1·03 | 1·33 |
| 23·25 lb. N/acre | 2·27 | 2·11 | 1·36 | 2·29 | 1·60 | 1·77 |
| 46·5 lb. N/acre | 2·35 | 2·26 | 1·43 | 3·09 | 1·86 | 2·08 |
| Weeds absent: | | | | | | |
| Control | 2·25 | 2·38 | 1·52 | 2·80 | 1·71 | 2·33 |
| 23·25 lb. N/acre | 3·35 | 3·42 | 1·88 | 3·56 | 2·27 | 3·11 |
| 46·5 lb. N/acre | 3·89 | 3·40 | 2·02 | 4·26 | 2·35 | 3·47 |

Although it was not possible to sample all the six trials when the crops were in the same stage of development, yet it seems clear from the data in Table IX that in spite of the presence of weeds, a considerable portion of the nitrogen added was absorbed by the crop. In the case of Holyport and Milverton, where—as at Dorchester and Hascombe—sampling was carried out during the period of tiller production, the rises in nitrogen content demonstrate that, in spite of weed competition, absorption of the added nitrogen had taken place within a short time of the application.

*Changes in the phosphorus and potassium contents.* In addition to the determinations of nitrogen content, the samples in four experiments were analysed for phosphorus and potassium. The results of these analyses are shown in Table X.

Table X. *The effect of nitrogenous manuring on the phosphorus and potassium content of the cereal in the presence and absence of weeds*

| Centre ... | Hascombe | | Holyport | | Dorchester | | Milverton | |
|---|---|---|---|---|---|---|---|---|
| Crop ... | Oats | | Oats | | Barley | | Barley | |
| Weed species ... | *R. raphanistrum* | | *R. raphanistrum* | | *R. raphanistrum* | | *R. raphanistrum* | |
| Date of sampling ... | 14. vi. 35 | | 4. vi. 35 | | 12. vi. 35 | | 29. v. 35 | |
| | $P_2O_5$ | $K_2O$ | $P_2O_5$ | $K_2O$ | $P_2O_5$ | $K_2O$ | $P_2O_5$ | $K_2O$ |
| | (Percentage of dry matter) | | | | | | | |
| Weeds present: | | | | | | | | |
| Control | 0·67 | 2·87 | 0·68 | 3·53 | 0·59 | 2·16 | 0·85 | 2·89 |
| 23·25 lb. N/acre | 0·79 | 3·68 | 0·76 | 4·98 | 0·65 | 1·79 | 0·87 | 3·17 |
| 46·5 lb. N/acre | 0·81 | 3·79 | 0·77 | 4·23 | 0·66 | 1·81 | 0·87 | 3·77 |
| Weeds absent: | | | | | | | | |
| Control | 0·70 | 3·32 | 0·89 | 4·21 | 0·68 | 2·83 | 0·84 | 3·84 |
| 23·25 lb. N/acre | 0·74 | 4·28 | 0·90 | 5·84 | 0·77 | 2·37 | 0·88 | 4·20 |
| 46·5 lb. N/acre | 0·75 | 3·59 | 0·89 | 5·87 | 0·78 | 2·32 | 0·80 | 4·39 |

Where no nitrogen has been added, the presence of *R. raphanistrum* has depressed the potassium content of the cereal in all four experiments. With the exception of the experiment at Dorchester, the addition of nitrogen to the weedy crop has raised the potassium level up to or above that of the weed-free crop receiving no nitrogen. The effect of weed competition or nitrogenous manuring on the phosphorus content is much less marked. In only one experiment (Holyport) is the difference in phosphorus content between the weedy and weed-free crop at all appreciable.

*The influence of water supply on the growth of weeds and cereals*

Earlier in the paper it has been pointed out that the years 1934 and 1935 differed largely in the spring rainfall. In Table XI the monthly rainfall figures are set out together with the average rainfall over the standard period of 1881–1915. Where rainfall figures were not obtainable adjacent to the experimental centre, the nearest recording station is given in brackets. During 1934 the rainfall in the eastern and southern counties was slightly above the average in March and April and below the average in June and more particularly May. In 1935, March was a dry month but April was exceptionally wet. During May the rainfall was normal while in June it was well above the average.

Table XI. *Monthly rainfall (in.)*

| Centre | | March | April | May | June |
|---|---|---|---|---|---|
| Jealott's Hill | 1934 | 2·22 | 1·59 | 0·53 | 1·27 |
| | 1935 | 0·40 | 3·00 | 1·76 | 4·05 |
| | Mean* | 1·87 | 1·52 | 1·72 | 2·13 |
| Dorchester | 1934 | 1·72 | 2·14 | 0·58 | 1·22 |
| | 1935 | 0·44 | 3·64 | 2·01 | 4·81 |
| | Mean | 1·66 | 1·51 | 1·75 | 2·02 |
| Milverton | 1934 | 3·05 | 2·89 | 1·78 | 1·57 |
| | 1935 | 1·18 | 4·85 | 1·52 | 2·76 |
| | Mean | 2·73 | 2·17 | 2·13 | 2·17 |
| Holyport (Maidenhead) | 1934 | 2·22 | 1·63 | 0·61 | 1·63 |
| | 1935 | 0·49 | 3·77 | 1·23 | 3·25 |
| | Mean | 1·87 | 1·52 | 1·72 | 2·13 |
| Bury St Edmunds | 1934 | 2·16 | 2·63 | 0·75 | 1·60 |
| | Mean | 1·89 | 1·53 | 1·82 | 2·10 |
| Broadclyst (Bramford Speke) | 1935 | 1·54 | 3·99 | 2·10 | 2·53 |
| | Mean | 2·34 | 2·01 | 1·82 | 1·85 |
| Hascombe | 1935 | 0·42 | 3·65 | 1·32 | 3·99 |
| | Mean | 2·41 | 1·84 | 2·00 | 2·12 |
| Ilminster | 1935 | 0·98 | 4·08 | 1·48 | 3·49 |
| | Mean | 2·45 | 2·06 | 2·00 | 2·03 |
| Medmenham (Henley-on-Thames) | 1935 | 0·55 | 3·66 | 1·66 | 5·00 |
| | Mean | 2·06 | 1·66 | 1·95 | 2·18 |

* For Jealott's Hill the average figures cited are those for Maidenhead.

Although in 1934 the crops at the majority of the centres showed no visible signs of water shortage, the effect of low rainfall both on the establishment and growth of the weeds was evident at several centres. This was particularly noticeable at Jealott's Hill; many mature plants of all the three species (*B. arvensis*, *R. raphanistrum* and *P. Rhœas*) were only a few inches high at maturity. This lack of an adequate moisture supply also resulted in a dying off of many plants, *vide* Table XII. No stunted growth was observed at those centres where rainfall approached the average, e.g. Milverton. In 1935 the growth of the weed in each experiment was luxuriant.

Table XII. *The effect of spring drought on the mortality of* Brassica arvensis *and* Raphanus raphanistrum *growing in oats*

| | Change in density (initial population expressed as 100) | | | | |
|---|---|---|---|---|---|
| | 20 April* | 6 May | 19 May | 7 June | 23 June |
| *B. arvensis* | 100 | 53·7 | 42·8 | 29·6 | 14·3 |
| *R. raphanistrum* | 100 | 73·7 | 42·1 | 28·4 | 24·3 |

* By 20 April germination had ceased, by 23 June all plants were in flower.

## Discussion

On the basis of the present experiments, conclusions can be drawn as to the nature of the competition between annual weeds and cereal crops. It has been demonstrated that the presence of *Brassica arvensis* in spring barley leads primarily to a decrease in the number of tillers and fertile shoots, while *Raphanus raphanistrum* in addition to some extent reduces the ear size. On the other hand competition between *R. raphanistrum* and spring oats may decrease the panicle size more than the number of fertile shoots. In four out of the six experiments which were observed in detail, nitrogen applied to the clean or weed-free crop produced similar effects. At Medmenham, Ilminster and Dorchester, the rise in the tiller production of the barley due to additional nitrogen was in direct contrast to weed competition. This led to a reduction. The opposite effects of nitrogenous manuring and weed competition indicate that nitrogen played an important part in the competition between the barley and the weed. That nitrogen supply controls tiller production in barley has been stressed by Gregory and his co-workers (1937) in their extensive investigations of its growth and metabolism under varying nutritional conditions. Although they showed that phosphorus and potassium deficiencies may also reduce the tiller number, yet at the much higher levels of these two elements occurring normally in English

arable soils, the nitrogen effect is predominant. Moreover, Richardson & Crowther (1935) have demonstrated that under field conditions tiller production is largely correlated with available nitrogen supply.

Further evidence as to the importance of nitrogen in weed competition is forthcoming from the experiment on oats at Hascombe. Here again the presence of *R. raphanistrum* had opposite effects to nitrogenous manuring on the development of the cereal. The addition of nitrogen increased equally shoot number and panicle size, while weed competition diminished both. Confirmatory evidence of the role played by nitrogen in the development of oats is scanty. In America, McClelland (1931) found that the response of spring and winter oats to nitrogen, phosphorus and potassium was variable. Nitrogen on the whole increased slightly tiller production, while phosphorus gave somewhat larger panicles. Only phosphorus however, increased the yield, while potassium caused a depression. The large effects of nitrogen in increasing both tiller number and panicle size found in the present experiments indicate that the conditions were very different from those under which McClelland conducted his experiments. Moreover, Williams (1936) working in Australia considered that except at very low phosphorus levels, the early growth of oats was limited by nitrogen supply.

In these four experiments the crop development data indicate that nitrogen is an important factor in weed competition. If it is postulated that competition between the crop and the weed is solely for nitrogen, then it follows that in nitrogen deficient soils, the depression in yield will be greater than in nitrogen rich soils. It would also be expected that the yield of a weedy crop could be raised by nitrogenous manuring to the same level as that of a clean crop provided that sufficient nitrogen is added to supply the maximum requirements of both the crop and the weed. The response of a weedy crop to nitrogen supplied will be dependent upon the relative amounts taken up by the crop and the weed. If the proportion absorbed by the weed is low, then the response of the crop with increasing nitrogen supply will at first be large. At higher nitrogen levels the response will gradually fall off when the amounts added are such that the nitrogen available to the crop no longer controls the yield. If, on the other hand, the proportion absorbed by the weed is large, then the rate of increase in crop yield will at first be very low. As more nitrogen is added, a point will be reached when nitrogen is no longer controlling the weed's growth and the proportion available to the crop above this point will rise. In consequence the rate of increase in crop yield will be higher at these than at lower nitrogen levels. Further

addition of nitrogen will lead finally to no further increases in crop yield. On the basis of these assumptions, the effects of increasing nitrogen supply on the yield of the cereal under the various conditions are shown in Fig. 5.

From the curves given in Fig. 5, it is possible to deduce what will be the effect of adding nitrogen to the crop in the presence and absence of weeds. If the quantity ($x$) is small, then the effect may be of two types. If there is a high relative absorption by the weed, then the initial difference in yield between the clean and the weedy crop may become greater

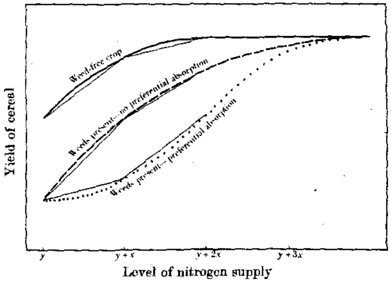


Fig. 5. Diagrammatic schema of the relationship between cereal yield and nitrogen supply in the presence and absence of weeds. The continuous line curve shows the effect of additional nitrogen on the yield of the weed-free crop. The broken line curve represents the realtionship between yield and nitrogen supply on the assumption that additional nitrogen is not absorbed preferentially by the weed and the "dotted" curve, the same relationship on the assumption that the added nitrogen is preferentially absorbed by the weed. The straight lines indicate the probable effects on yield of adding nitrogen at the rates of $x$ and $2x$ under the various sets of conditions.

through the addition of nitrogen. If on the other hand, there is a high relative absorption by the crop, the initial difference may remain constant or become less. The application of a second unit of nitrogen ($2x$) will lead to the difference in yield between the clean and weedy crop being less than at the lower level. The response of the weedy crop to this second quantity of nitrogen may be, however, larger or smaller than to the first according to whether the added nitrogen is or is not preferentially absorbed by the weed.

Considering the yield results in the light of the foregoing postulates there is evidence from several experiments that competition between the weed and the cereal was solely for nitrogen. If other factors than

nitrogen had played a principal role, then the addition of nitrogen to the weedy crop would not, as in fact it did, have raised the yield level to or above that of the weed-free crop (*Medmenham, Ilminster, North Petherton*, Broadclyst, Dorchester and Hascombe). At Hascombe there is, however, evidence that at the highest level of nitrogen supply, competition was only in part for nitrogen, since while in the clean crop there was a linear response to additional nitrogen, in the weedy crop there was a significant falling off in response to the higher rate of application.

In five experiments, the results cannot be explained satisfactorily on the assumption that nitrogen is the sole factor governing competition between the weed and the crop. In three experiments (Holyport (1934) and Milverton (1934 and 1935)) nitrogen applied either to the clean barley or barley containing *R. raphanistrum* did not increase the crop yield. At Bury St Edmunds, while the weed-free crop responded to the application of nitrogen, the barley containing *B. arvensis* did not. It might be put forward that the weedy crop did not respond to the added nitrogen in these cases on account of the preferential absorption of the nitrogen by the weed. This is in direct conflict with the evidence obtained in 1935 on the uptake of nitrogen by the weedy crop. Even at Milverton and Holyport, where the density of *R. raphanistrum* was higher than in any of the other experiments, the application of nitrogen to the weedy crop brought about striking increases in the nitrogen content of the cereal. For example at Holyport nitrogen applied at single and double rates to the weedy crop raised the nitrogen content from 1·33 to 1·77 and 2·08 % respectively. Yet in spite of these increases, there was only a small rise in the level of yield for nitrogen at the *single* rate. At the double rate of application, even though the nitrogen content was of the same order as that of the weed-free crop, yet the yield was some 29 % less.

While at Holyport the response of the weedy crop to nitrogen at only the single rate demonstrates that competition was in part for nitrogen, the data obtained at Milverton indicate that here nitrogen played only a minor role. Even though at the time of tillering the nitrogen content of the weedy crop, top dressed with nitrogen, was equal to that of the clean barley, yet the production of tillers was markedly inferior. The only effect of additional nitrogen in the weedy crop was to increase by a small extent the proportion of tillers that became fertile shoots. Furthermore although the ear size was some 30 % less than in the clean crop, it was not, as in the weed-free crop, increased by nitrogenous manuring.

In discussing earlier in the paper other factors than nitrogen supply which might be expected to play a part in weed competition, it was pointed out that in view of the known small response obtained when phosphorus or potassium compounds are added to spring cereals it seemed unlikely that competition would be associated with a deficiency in these two essential elements. The data given in Table X support this view. In all the experiments, irrespective of whether nitrogenous manuring did or did not raise the yield of the weedy crop to the level of the clean crop, the phosphorus and potassium contents of the cereal showed similar trends. The phosphorus content, except for the small differences at Holyport, was not appreciably affected either by nitrogenous manuring or the presence of weeds. With the exception of the experiment at Dorchester increasing nitrogen content was associated with a rise in potassium. Similar increases in the potassium content of oats following on nitrogenous manuring have been reported by Blank & Giesecke (1934), while Voigt *et al.* (1934) have recorded in addition that with excessive nitrogen supplies the potassium content again falls. Both at Holyport and Milverton, in spite of the large differences in the yield, the phosphorus and potassium contents of the weedy crop heavily manured with nitrogen were of the same order as those in the clean crop receiving no nitrogen.

In attempting to assess the importance of the water supply in relation to weed competition no direct evidence can be obtained from these experiments. It has been shown (*vide* Table XI) that in the spring of 1934 the rainfall was in general below the average, while in 1935 it was above. It is possible that in 1934 there was competition for water between the crop and the weed at some centres. For example the lack of response of the clean crop at Holyport to nitrogenous manuring in spite of the low level of yield, suggests that water shortage intensified by the presence of the weeds may have played some part in weed competition. Similarly, the fact that the weed-free barley alone responded to nitrogen at Bury St Edmunds indicated that in the weedy crop growth was controlled not by nitrogen but by some other factor, possibly water. On the other hand, the luxuriant growth of *B. arvensis* at this centre in contrast to the stunted growth of *R. raphanistrum* at Holyport[1] and *B. arvensis* at Jealott's Hill did not suggest a water deficit. In the 1935 experiments the failure of added nitrogen to increase the yield of the weedy crop at Milverton and Holyport can hardly be attributed to a

[1] The stunted growth at this centre may in part have been due to attack by flea beetles (*Phyllotreta undulata* and *Plectroscelis concinna*).

water shortage. Over the period when the intensity of competition was greatest, the rainfall was equal to or above the average as in the other 1935 experiments. In these, nitrogenous manuring did in contrast have a marked effect on the yield of the weedy crop. Moreover, Cochran in Russell & Voelcker's recent memoir (1936), examining statistically the effect of spring rainfall on the yield of barley at Woburn, reached the conclusion that even in this light soil excess spring rainfall tended to decrease the yield.

Apart from competition for nutrients and water there remains the possibility of competition for light. As in the case of water, direct evidence of such competition is difficult to obtain in field experiments. The data set out in Table VIII indicate that apart from the period just after germination, the heights of *B. arvensis* and *R. raphanistrum* keep pace until the flowering stage is reached with the height of the crop. Although when the density of the weed is low its presence may not to any great extent reduce the amount of light falling on the lower half of the cereal, a high weed density reduces the light intensity appreciably. In this connexion it is of interest to note that although weed competition may have such marked effects on tillering and ear size, the height of the cereal is little affected, especially at the lower nitrogen levels. This suggests that up to a point shading of the lower half of the cereal can be compensated for by stem elongation. When, however, the density of the weed is high and the shading marked, competition for light may become operative. It is significant that at both Milverton and Holyport, where neither competition for nutrients nor competition for water provides satisfactory explanation of the effects observed in 1935, the weed density was very much higher than in the other experiments. In both experiments *R. raphanistrum* formed a dense tangled mass and the cereal exhibited signs of apparent light shortage. Compared to the clean crop there was a premature dying back of the older leaves, while the stems appeared thin and chlorotic. Some evidence as to the weakness of the straw is given by the Holyport data in Table VII. Where the weed was removed at the time of flowering severe lodging of the crop subsequently occurred. In contrast little lodging occurred in both the weed-free plots and those in which the untouched mass of weed supported the crop. This severer lodging is reflected in the significant yield differences between the plots in which the weed was removed and those in which it was left undisturbed. Although to the authors it seems improbable, it might be suggested that this reduction in yield could be ascribed to damage sustained by the crop when the weeds were hand pulled.

In the first section of this paper, it has been demonstrated that the intensity of weed competition varies with the species. On an equal density basis *R. raphanistrum* brings about a greater reduction in crop yield than *B. arvensis*, while this in turn has a much more marked effect than *P. Rhœas*. In the case of *R. raphanistrum* and *B. arvensis* it has been seen that in a normal year competition between these weeds and the cereal is for nitrogen and possibly light when the density is high. On the basis of these findings it is not surprising that competition from *P. Rhœas* has little effect on the cereal. In the first place the seeds of *P. Rhœas* germinate considerably later in the spring than those of either *R. raphanistrum* or *B. arvensis*. In the second place on account of the smallness of the seed[1] the seedlings do not reach any considerable size until the end of May or early June. Furthermore until the flowering shoots are formed the plant is of a rosette form and is always overshadowed by the crop. In consequence, during the early development of the cereal when weed competition is most deleterious, the plants of *P. Rhœas* are too small to compete either for nitrogen or for light. Only in winter cereals where *P. Rhœas* has germinated in the autumn should competition in the following spring reduce the crop yield.

In conclusion, the results of this investigation have revealed some points of practical importance to the farmer. In a year of normal rainfall it would appear that when the weed species is low growing or, in the case of the taller kinds, when the density is not too great, then competition with the crop is largely for nitrogen. Under such conditions applications of nitrogen to the weedy crop may give very considerable increases in yield. The magnitude of these increases will be dependent on the amount of nitrogen applied, the available nitrogen in the soil and the proportion absorbed by the weed. Under some conditions the increases in yield obtained from a given quantity of nitrogen may be markedly *greater* in the weedy than in the weed-free crop, as for example at Hascombe. If the average over the two seasons is taken for the ten experiments in which the weed density was not excessive then nitrogen applied at the rate of 23·25 lb./acre gave increases of 3·3 and 2·5 cwt./acre in the clean and weedy crop respectively. In 1935, a more favourable season for cereals, applications of 23·25 and 46·5 lb. N/acre raised the yield (average of four experiments) by 4·2 and 6·1 cwt./acre in the clean crop and 4·3 and 7·0 cwt. in the weedy crop. High nitrogenous manuring

[1] In the samples of seed collected for use in this investigation the average seed weight of *P. Rhœas* was 0·00014 g. as against 0·0036 and 0·0025 g. for *R. raphanistrum* and *B. arvensis* respectively.

may therefore be more economic than weed suppression when the weed population is not too great. If weed control is necessary on account of a high weed density, the operation should not be delayed, for it is in the early stages that "the noisome weed does without profit suck the soil's fertility from" cereal crops.

## Summary

In continuation of earlier work, a study has been made of the factors controlling the competition between spring-sown cereals and annual weeds. During the years 1934 and 1935 some eighteen replicated trials were carried out in widely different localities. In six experiments, observations involving some 20,000 counts were made on the cereal development. Competition between *Brassica arvensis* (yellow charlock) and spring barley primarily reduced the number of tillers and fertile shoots; competition on the other hand with *Raphanus raphanistrum* (white charlock) diminished in addition ear size. The presence of *R. raphanistrum* in spring oats may decrease panicle size to the same extent as shoot number. In four out of six experiments the addition of nitrogen to both the weedy and clean crop had similar effects, greatly increasing tiller production in barley and both panicle size and shoot number in oats.

In six experiments nitrogenous manuring raised the yield of the weedy crop (containing either *B. arvensis*, *R. raphanistrum* or *Chrysanthemum segetum* (corn marigold)) to or above the level of the clean crop; in four the yield increases were greater in the weedy than in the weed-free crop. In one experiment doubling the amount of nitrogen applied to the weedy crop did not increase the yield of oats more than the single rate, although the final yield level was considerably below that of the weed-free oats. In four other experiments additional nitrogen did not raise the yield of the weedy crop; but in only one was the yield of the clean crop increased by nitrogenous manuring.

The presence of weeds depressed the nitrogen and potassium contents of the cereal but did not affect the phosphorus content. Additional nitrogen counteracted the effects of weed competition; raising both the nitrogen and potassium contents.

The development of the weed species investigated was related to the spring rainfall. In the dry spring of 1934, the establishment of the seedlings and their later growth was adversely affected. In the wet spring of 1935, subsequent to the seedling stage and until the flowers were in full bloom, the height of either *B. arvensis* or *R. raphanistrum* kept

pace with the height of the crop. When the density of *R. raphanistrum* was high, there was a tendency for the cereal to develop a weak straw.

The intensity of the weed competition was dependent upon the weed species. *R. raphanistrum* was more aggressive than *B. arvensis* which in turn brought about greater depressions in yield than *P. Rhœas* (field poppy). On the other hand, *R. raphanistrum* was attacked more severely than *B. arvensis* by flea beetles (*Phyllotreta undulata* and *Plectroscelis concinna*).

It is concluded that in a year of normal rainfall competition between the crop and the weed is principally for nitrogen and light. The light factor is, however, only operative when the weed species is tall growing and the density is high. In the majority of cases competition is solely for nitrogen, while the critical period is confined to the early stages in the development of the cereal. Weeds such as *R. raphanistrum* capable of making rapid growth during this period depress the crop yield to a greater extent than those which develop later in the season. Where competition is largely for nitrogen it may be more economic to raise the yield of the weedy crop by nitrogenous manuring rather than by weed suppression.

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# STUDIES IN SOIL CULTIVATION

## VIII. THE INFLUENCE OF THE SEED BED ON CROP GROWTH

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(With Two Text-figures)

### I. Introduction.

In the preceding paper it has been shown that on the whole crop yields are surprisingly insensitive to cultivation. In this paper the effect of different seed beds on the growth of the crop will be examined. Keen and his co-workers (1930) published an account of the first experiments made at Rothamsted on the use of rotary cultivation as a method of preparing a seed bed. These experiments were carried out in the harvest years 1926–9, and in each case the rotary cultivator was working on a stale furrow in the spring. The main conclusions reached were that the germination of the crop on the rototilled land was more rapid than on the ploughed and harrowed land, that the yield of barley was about the same under both treatments, but the yield of swedes was lower on the rototilled land.

Three main extensions have been made since 1929: using the Rototiller as the sole means of preparing a seed bed instead of using a stale furrow, using two working depths, and introducing the cultivator or grubber[1] as an alternative method of preparation for the seed bed. The principal object of this paper is to follow the crop and weed growth as closely as possible on seed beds prepared by these different methods.

### II. The methods used to prepare the seed beds

The plough and harrows have been taken as the standard method for preparing the seed bed. The plough cuts the furrow slice, turns it over and crumbles it, and finally the end of the mouldboard may compress it, and so reduce the comminution produced in turning the

[1] For the reasons given in the footnote on p. 213 of the preceding paper we are using the name grubber instead of cultivator throughout.

slice. By altering the shape of the mouldboard the crumbling or the compression can be increased. This furrow slice is left to weather if need be and is then worked to bring it to a suitable fineness for the seed bed.

The rotary cultivator used[1] had its tines mounted on a single rotating horizontal shaft. These break up, churn and mix the soil fairly thoroughly throughout the whole depth of cultivation, leaving a fairly fine but loose seed bed. It thus is able to produce a seed bed in one operation which is invaluable if rate of working is important. Culpin (1936*a*) has urged that this must involve an extravagant use of power because there is no need to do all the breaking of the soil for the weather will do much comminution of a furrow slice. In the absence of suitable measurements no test of this hypothesis can yet be made. There is, however, evidence to suggest that Culpin's criticism is false, for a tractor drawing a rotary cultivator seems to cover a larger area of land per hour than if it is drawing a plough, so presumably the preparation of a seed bed in one operation by a rotary cultivator would need less fuel than several ploughings.

The cultivator or grubber breaks the soil up without turning a furrow slice and without much mixing of the soil from different depths. The action of the rigid-tined grubber closely resembles the primitive method of ploughing, which is still largely followed in the East. Von Nitzsch (1935, 1936*b*) in Germany claims that in suitable soil conditions a tractor-drawn grubber fitted with properly mounted and designed tines can produce more quickly, and with a lower power consumption, as good a seed bed as one prepared by the plough.

The one operation the plough can do that these cultivators cannot do is to bury material, either dung or weeds. If land is not ploughed for several years in succession perennial weeds tend to increase in number, so that these methods are not capable of displacing the plough completely, though they can displace it if the land is reasonably clean.

## III. Quantitative measures of the results produced by the cultivation implements on the soil

Two methods have been employed in measuring the type of work done by these cultivation implements. The first uses the weed seeds in the soil as indicators to show how the soil layers at different depths are mixed together. The number of weed seeds in 24 cu. in. of soil is taken,

[1] A Rototiller kindly supplied by Messrs Geo. Munro, Ltd. of Waltham Cross was used throughout these experiments.

by the method to be described later in this paper, at successive 2 in. depths down to the bottom of the cultivation layer, both before and after cultivation. Table I gives the distribution of these weed seeds with depth in the soil and shows clearly that ploughing leaves the majority of the weed seeds near the bottom of the furrow, thus indicating, as is well known, that the plough brings the soil at the bottom of the furrow up to the top and buries the surface soil at the bottom. The Rototiller, on the other hand, distributes them more evenly, indicating that it mixes the soil from different depths together fairly thoroughly throughout the greater part of its working depth.

Table I. *Distribution of weed seeds in the soil profile before and after cultivation*

| Depth in inches from which sample was taken ... | Implements working to 8 in. depth | | | | Implements working to 4 in. depth | |
|---|---|---|---|---|---|---|
| | 0–2 | 2–4 | 4–6 | 6–8 | 0–2 | 2–4 |
| Percentage distribution of weed seeds: | | | | | | |
| Before cultivation | 38·6 | 23·9 | 21·9 | 15·6 | 64·0 | 36·0 |
| After ploughing | 15·2 | 22·1 | 33·2 | 29·5 | 38·4 | 61·6 |
| After rototilling | 30·8 | 26·2 | 25·2 | 17·8 | 49·8 | 50·2 |

The second method measures the comminution of the soil produced by the implement and consists in putting soil samples taken before and after cultivation on a bank of sieves, sieving gently, and determining the size distribution of the soil aggregates (Keen *et al.* 1930; Keen, 1933). The proportions of soil aggregates retained on $1\frac{1}{2}$ in. (38 mm.), $\frac{5}{8}$ in. (16 mm.), $\frac{1}{4}$ in. (6·4 mm.) and 3 mm. sieves and passing the 3 mm. sieve are determined. The results can be expressed as the percentage of soil on the different sieves and given in tabular or preferably in graphical form both before and after the soil has been cultivated. Alternatively, if a single value is needed an estimate may be made of the total surface of the soil crumbs both before and after cultivation, or the ratio of the percentage of soil passing a given sieve before and after cultivation can be given.

Table II. *Size distribution of soil crumbs after cultivation* (*Long Hoos*, 1934)

| Crumb size | Before cultivation | Plough Deep | Plough Shallow | Plough Mean | Grubber Deep | Grubber Shallow | Grubber Mean | Rototiller Deep | Rototiller Shallow | Rototiller Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| Larger than $1\frac{1}{2}$ in. | 13·2 | 28·2 | 18·5 | 23·3 | 10·6 | 7·8 | 9·2 | 4·9 | 5·5 | 5·2 |
| $1\frac{1}{2}$–$\frac{5}{8}$ in. | 14·9 | 12·9 | 14·3 | 13·6 | 15·2 | 13·2 | 14·2 | 9·9 | 14·0 | 11·9 |
| $\frac{5}{8}$–$\frac{1}{4}$ in. | 17·1 | 14·1 | 16·2 | 15·2 | 14·0 | 15·3 | 14·7 | 14·7 | 16·2 | 15·5 |
| $\frac{1}{4}$ in.–3 mm. | 27·5 | 24·1 | 26·4 | 25·3 | 30·9 | 31·1 | 31·0 | 33·9 | 31·7 | 32·8 |
| Passing 3 mm. | 27·3 | 20·7 | 24·6 | 22·6 | 29·3 | 32·6 | 30·9 | 36·6 | 32·6 | 34·6 |

Table II gives an example of the comminution produced by the plough, the grubber, and the Rototiller working at a depth of either 8 in. (deep) or 4 in. (shallow) on barley stubble. To work to 8 in. with the grubber or Rototiller it was necessary to go over the land twice.

For the Rothamsted soil the most useful single figure to replace the five used to specify the distribution curve appears to be the proportion of soil passing the ⅝ in. sieve. The ratio of the weight passing after cultivation to that passing before gives a measure of the comminution or production of aggregates by the implement according as the ratio is greater than or less than unity. Table III gives the comminution produced by the various cultivation implements expressed on this basis.

Table III. *Comminution of soil aggregates larger than ⅝ in. produced by cultivation*

(1) Little Hoos, 1930:

| | Plough | Pulverator plough* | Rototiller |
|---|---|---|---|
| Directly after cultivation | 1·12 | 1·22 | 1·59 |
| Seed bed just before drilling | 1·41 | 1·53 | 1·63 |

The plough and the pulverator plots received various harrowings and discings after the main cultivation to get the seed bed. The rototilled land had no subsidiary cultivations.

(2) Pastures, 1932:

| | Plough | Rototiller | Mean |
|---|---|---|---|
| Working to 8 in. | 1·31 | 2·46 | 1·89 |
| Working to 4 in. | 1·47 | 2·11 | 1·79 |
| Mean | 1·39 | 2·29 | — |

(3) Long Hoos, 1933:

| | Plough | Grubber | Rototiller | Mean |
|---|---|---|---|---|
| Working to 8 in. | 1·18 | 1·10 | 1·43 | 1·24 |
| Working to 4 in. | 1·11 | 1·05 | 1·19 | 1·12 |
| Mean | 1·14 | 1·07 | 1·31 | — |

(4) Long Hoos, 1934:

| | Plough | Grubber | Rototiller | Mean |
|---|---|---|---|---|
| Working to 8 in. | 0·84 | 1·17 | 1·18 | 1·06 |
| Working to 4 in. | 0·90 | 1·07 | 1·14 | 1·04 |
| Mean | 0·87 | 1·16 | 1·12 | — |

* For a description of this plough see the footnote on p. 214 of this issue.

The main conclusions to be drawn from this table are:

(1) The great variability of the results from year to year.

(2) The grubber and Rototiller always comminute the soil, the plough only does so in certain seasons.

(3) The grubber and Rototiller produce better comminution with deep than with shallow working, which is self-evident as they go over the land twice for deep working.

(4) *The Rototiller usually comminutes the soil more than the grubber and the grubber more than the plough.*

*A further result that has emerged, but is not shown up in any of the tables, is that in every year that the Rototiller has been used it has left* a seed bed. On the other hand, it has sometimes taken many subsequent operations to obtain a seed bed from the ploughed land, the most difficult year being 1930 when no less than six were required to get some of the ploughed plots into a suitable condition for sowing.

Various other methods have been proposed to determine the type of work done by the cultivation implements preparing the seed bed. Culpin (1936*b*), for example, has measured the loosening produced by a plough and a Gyrotiller by penetrometer methods. Apsits (1936) and von Nitzsch (1936*a*, *b*) both measure the pore-space of the soil before and after cultivation. In fact von Nitzsch (1936*b*) claims that for every 1 % increase of pore-space during the growing season the yield usually increases by about 2½–3 %, and in eight of his experiments when he increased the pore-space by 5·4 %, during the growing season he increased the yield by 26 %. Unfortunately none of these methods can be used at Rothamsted at present owing to the number of stones in the soil. But other methods for the analysis of the tilth produced by different implements, which will work in the presence of stones, are being developed here.

## IV. Effect of cultivation on plant growth

A description of the experiments and of their design has already been given in the preceding paper. Detailed measurements have been made on the following experiments:

| Crop | Field | Season | Measurements taken by |
|---|---|---|---|
| (1) Winter wheat | Little Hoos | 1930–1 | R. J. Kalamkar |
| (2) Winter wheat | Pastures | 1932–3 | Staff of Soil Physics Dept. |
| (3) Winter wheat | Pastures and Long Hoos | 1933–4 | N. P. Mehta |
| (4) Spring barley | Long Hoos | 1934 | N. P. Mehta |
| (5) Mangolds | Long Hoos | 1934 | N. P. Mehta |

### *Germination*

The germination on the rototilled plots is more rapid than on the other treatments studied (plough, pulverator-plough, and grubber) for the first few days. Then the other treatments usually catch up, and when germination is complete there is no systematic difference in favour of any treatment. A typical result is given in Fig. 1. This result has been

found in six out of the seven germination counts made here. The only exception has been the 1934 mangold experiment when germination was both faster and better on the ploughed than on the rototilled plots. It is unlikely that it is a peculiarity of the mangold plant, for in 1926 and 1928 Keen *et al.* (1930) found that swedes germinated more rapidly on the rototilled than on the ploughed plots. It is more likely due to weed competition, for the bean stubble was left over the winter and was foul

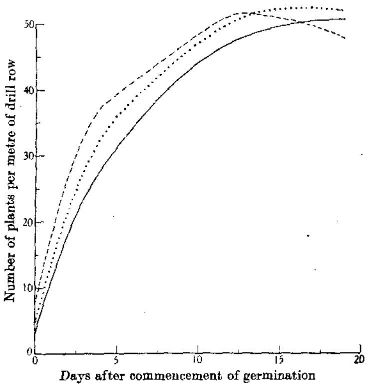


Fig. 1. Rate of germination of wheat on different seed beds (Long Hoos, 1933)

——— Ploughed plots. ········· Grubbed plots. - - - - - - - - Rototilled plots.

with weeds when cultivated in the spring. The plough was able to bury the weeds, but the cultivators left many on the surface. Just before germination started, a rough estimate was made of the proportion of land covered by weeds. Table IV shows that the rototilled and grubbed plots were definitely dirtier and had a lower number of mangold plants than the ploughed plots, and the plots on which the cultivators worked shallow were dirtier and had a smaller plant number than those on which they worked deep. But although the rototilled plots appear to be cleaner than the grubbed plots this difference does not appear in the plant numbers.

In the three cereal crops on which observations were made, the plants

Table IV. *The relation between the weediness of the land and the germination of the mangold plants (Long Hoos,* 1934)

| | Percentage of land covered by weeds | | | Number of plants per metre length when germination was complete | | |
|---|---|---|---|---|---|---|
| | *Deep* | *Shallow* | *Mean* | *Deep* | *Shallow* | *Mean* |
| Plough | 12 | 17 | 15 | 17·6 | 17·8 | 17·7 |
| Rototiller | 36 | 61 | 48 | 13·1 | 10·8 | 11·9 |
| Grubber | 58 | 68 | 63 | 14·1 | 11·3 | 12·7 |
| Mean | 35 | 48 | — | 14·9 | 13·3 | — |

germinated more rapidly on the shallow than on the deep plots, but after the first few days the deep plots contained the same number or even slightly more plants. A typical result is given in Fig. 2 which shows the effect of the depth of ploughing on the rate of germination of wheat. No such effect was shown in the mangolds.

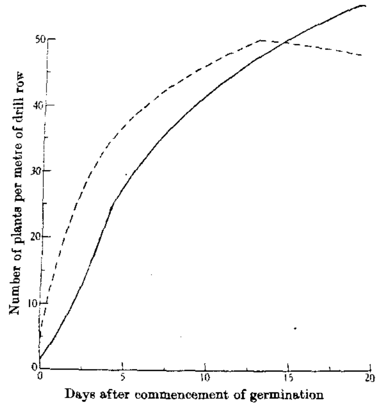


Fig. 2. Effect of depth of ploughing on the rate of germination of wheat (Long Hoos 1933).
——— Deep-ploughed plots. - - - - - - - - Shallow-ploughed plots.

*Plant number, shoot number, and tiller number*

The effect of the cultivation treatment on the number of winter wheat plants per metre of drill row during the winter and early spring is variable. On Little Hoos in 1930–1, when all the cultivations left the

land in very bad condition, the ploughed plots had not only fewer plants than those rototilled, but also a larger proportion of these plants were lost during the winter. In Pastures 1932–3 the ploughed plots had more plants than those rototilled, but the depth of tillage was unimportant, while on the same plots in 1933–4 the deep tilled plots had more plants than those shallow tilled. Certain general conclusions do, however, seem to emerge. Plots tilled to 8 in. depth usually have more plants than those tilled to only 4 in., and there is a possibility of low plant numbers on the shallow-grubbed and shallow-rototilled plots. As an example of this, the number of wheat plants per metre of drill row at the end of November 1934 on some Long Hoos plots was:

| | Ploughed | Rototilled | Cultivated |
|---|---|---|---|
| Deep tillage | 51·1 | 50·5 | 44·7 |
| Shallow tillage | 52·5 | 43·5 | 35·2 |

The effect of the cultivations on the number of barley plants in the spring of 1934 on the same plots was negligible.

The effect of cultivation on the number of shoots of winter wheat and spring barley has not been consistent. The results are given in summary form in Table V. In this table, 1 denotes the treatment with the highest number of shoots per metre and 6 the treatment with the lowest number. When the figure is enclosed in brackets it signifies that the number of shoots in this treatment is markedly less than that in the treatment denoted by the next lower figure, e.g. in the fourth line (3) is definitely less than 2 and (4) than (3).

Table V. *The effect of the seed bed preparation on the shoot number per metre for wheat and barley*

| | | | Deep | | | Shallow | | |
|---|---|---|---|---|---|---|---|---|
| | | | Plough | Rototiller | Grubber | Plough | Rototiller | Grubber |
| Wheat: | | | | | | | | |
| 1930–1 | Little Hoos | March | — | — | — | (2) | 1 | — |
| | | June | — | — | — | 1 | 2 | — |
| 1932–3 | Pastures | April | 2 | 4 | — | (3) | 1 | — |
| 1933–4 | Pastures | April | 1 | 2 | — | (3) | (4) | — |
| | | July | 2 | 1 | — | 3 | 4 | — |
| | Long Hoos | April | 2 | 4 | (3) | 1 | 6 | 5 |
| | | July | 3 | 5 | 2 | 1 | 6 | 4 |
| Barley: | | | | | | | | |
| 1934 | Long Hoos | May | 2 | 3 | (6) | 1 | 5 | 4 |
| | | July | 1 | 3 | 4 | 2 | 4 | 6 |

Thus for both wheat and barley in 1934 the ploughed plots in general had more shoots than the other treatments and the shallow-rototilled and shallow-grubbed plots tended to have the fewest, but this cannot be taken as anything more than a general statement.

The difficulty experienced in counting plants after they have tillered prevents precise conclusions being drawn on the effect of these cultivations on tillering. The general conclusion, however, is in reasonable accord with the well-known fact that the fewer the plants the more they tiller. There do not seem to be any specific cultivation effects on tillering.

Accurate plant counts were available in Pastures Field throughout the season 1933–4 as all the plants in each sampling area were uprooted. This field had a very poor stand of wheat, the average number of wheat plants per metre at the beginning of April being under 20, which is about half the number present in a normal crop. At the end of May, when the number of shoots had reached their maximum, the number of plants per metre varied with treatment, but the maximum number of shoots per plant was almost independent of treatment:

| | Plants per metre | | | Shoots per plant | | |
|---|---|---|---|---|---|---|
| | Plough | Rototiller | Mean | Plough | Rototiller | Mean |
| Deep | 14·1 | 13·2 | 13·6 | 2·31 | 2·28 | 2·29 |
| Shallow | 11·8 | 10·2 | 11·0 | 2·25 | 2·30 | 2·28 |
| Mean | 13·0 | 11·7 | — | 2·28 | 2·29 | — |

At the end of June the number of plants was unaltered, but the number of shoots had fallen and there was no further change in the shoot number at the end of July. The number of shoots per plant, averaged over these two counts, was:

| | Plough | Rototiller | Mean |
|---|---|---|---|
| Deep | 1·80 | 1·83 | 1·81 |
| Shallow | 1·79 | 1·98 | 1·89 |
| Mean | 1·79 | 1·91 | — |

so that the plots with the lowest plant number, namely the shallow-rototilled plots, were able to retain a larger number of shoots per plant, though the plants were not able to make more shoots than those on plots carrying a larger plant number.

*Crop height and miscellaneous measurements on wheat and barley*

The shoot height was measured on three crops, the wheat on Little Hoos, 1930–1, on Long Hoos, 1933–4, and the barley on Long Hoos, 1934. The differences in shoot height are small but in 1934 for barley were significant. On the whole, the crop is a little shorter on the shallow-rototilled and shallow-grubbed plots than on the others. The height of the barley shoots in inches during July, 1934 was:

| | Ploughed | Rototilled | Grubbed |
|---|---|---|---|
| Deep | 26·6 | 27·1 | 27·0 |
| Shallow | 26·8 | 25·8 | 26·0 |

Eye estimates made in 1935 and 1936 on the barley and wheat tend to confirm the result that there is a tendency for the shallow-rototilled and shallow-grubbed plots to be shorter than the rest, and sometimes the ploughed may be a little taller than the deep-rototilled and deep-grubbed plots.

The number of ears per shoot seems to be independent of cultivation treatment.

In the middle of July 1933 an eye estimate was made on Pastures Field of the ripeness of the wheat and no difference was found between the ploughed and rototilled plots. At the time of harvest there has only once been a note—in 1936—that the barley on the ploughed plots was dead ripe, that on the deep-rototilled and deep-grubbed was not quite so ripe, while there were many green ears on the shallow-rototilled and shallow-grubbed plots.

It is possible this last result is fairly general even when not visible to the eye. The moisture content of the flour made from the wheat grown on the Long Hoos experiment in 1933–4 was determined. The results, expressed as percentage loss of weight when the flour is dried at 110° C. overnight, were:

| | Plough | Rototiller | Grubber | Mean |
|---|---|---|---|---|
| Deep tillage | 12·78 | 12·90 | 13·47 | 13·05 |
| Shallow tillage | 13·32 | 13·57 | 13·50 | 13·46 |
| Mean | 13·05 | 13·24 | 13·48 | — |

The grain from the grubbed and the shallow-rototilled plots was the dampest, from the deep-ploughed and deep-rototilled the driest, and from the shallow-ploughed intermediate. These three differences are statistically significant. The probable interpretation of this result is that it shows the order of the ripeness of the wheat with the various treatments, the deep-ploughed and deep-rototilled being the ripest and the grubbed and shallow-rototilled the most immature. If this interpretation is correct it supports the result that maturity is delayed on the shallow-rototilled and shallow-grubbed plots. This effect did not show up on the barley of this year presumably because it was left long enough for all the grains to mature.

*Analysis of the yield of cereals*

The three crops for which a reasonable amount of data are available are the wheat on Pastures and the wheat and barley on Long Hoos for the season 1933–4. An analysis of the wheat yield is given in Table VI. The plant density, the yield per plant and per shoot are all higher for the wheat plants on Long Hoos than for those on Pastures. Hence, although there were fewer plants on Pastures, and although they had

more tillers than had those on Long Hoos, they yielded less: they were fewer in number, and poorer plants. The yield per shoot both on Pastures and on Long Hoos tended to be less on the shallow than the deep-tilled plots. On Pastures the yield per shoot was also definitely lower on the rototilled than on the ploughed plots, but there was no difference between them on Long Hoos. Hence on Long Hoos, where the yield was normal (23 cwt./acre of grain), the effect of the cultivation implement on the yield was mainly through differences in shoot number on the different treatments, while on Pastures, where the yield was subnormal (11 cwt./acre of grain), the effect was mainly through the plant number though it possibly also had a small effect on the yield of the individual plants.

Table VI. *Analysis of wheat yield. Long Hoos and Pastures*, 1934

| | Pastures | | | Long Hoos | | | |
|---|---|---|---|---|---|---|---|
| | Plough | Rototiller | Mean | Plough | Rototiller | Grubber | Mean |
| *Approximate number of plants per metre in July:* | | | | | | | |
| Deep | 13·3 | 13·9 | 13·6 | 24·2 | 24·1 | 27·0 | 25·1 |
| *Shallow* | *11·7* | *9·9* | *10·8* | *26·7* | *20·2* | *20·7* | *22·5* |
| Mean | 12·5 | 11·9 | — | 25·5 | 22·1 | 23·8 | — |
| Number of shoots per metre in July: | | | | | | | |
| Deep | 23·0 | 25·9 | 24·4 | 38·0 | 33·2 | 38·9 | 36·7 |
| Shallow | 21·5 | 19·3 | 20·4 | 39·8 | 30·3 | 35·6 | 35·2 |
| Mean | 22·2 | 22·6 | — | 38·9 | 31·8 | 37·2 | — |
| Mean yield of grain in grams per plant: | | | | | | | |
| Deep | 1·61 | 1·55 | 1·58 | 1·83 | 1·66 | 1·64 | 1·71 |
| Shallow | 1·60 | 1·53 | 1·56 | 1·65 | 1·73 | 1·96 | 1·78 |
| Mean | 1·60 | 1·54 | — | 1·74 | 1·70 | 1·80 | — |
| *Mean yield of grain in grams per shoot:* | | | | | | | |
| Deep | 0·93 | 0·83 | 0·88 | 1·17 | 1·20 | 1·14 | 1·17 |
| *Shallow* | *0·87* | *0·79* | *0·83* | *1·11* | *1·15* | *1·14* | *1·13* |
| Mean | 0·90 | 0·81 | — | 1·14 | 1·17 | 1·14 | — |
| Grain-straw ratio: | | | | | | | |
| Deep | 0·78 | 0·84 | 0·81 | 0·83 | 0·83 | 0·85 | 0·84 |
| Shallow | 0·78 | 0·84 | 0·81 | 0·85 | 0·82 | 0·85 | 0·84 |
| Mean | 0·78 | 0·84 | — | 0·84 | 0·82 | 0·85 | — |
| Yield of grain in cwt./acre: | | | | | | | |
| Deep | 12·1 | 12·2 | 12·1 | 25·1 | 22·6 | 25·1 | 24·3 |
| Shallow | 10·6 | 8·6 | 9·6 | 25·0 | 19·8 | 23·0 | 22·6 |
| Mean | 11·3 | 10·4 | — | 25·0 | 21·2 | 24·1 | — |

A further analysis of yield of the wheat on Long Hoos was made. The effect of plant number, shoot number, shoot height, weed number and the number of grains per earhead on each other and on the yield of grain and straw was made when both the effects of soil heterogeneity and the different experimental treatments had been eliminated. The only effect

found was that the yield of grain was dependent on the shoot number, which confirms in detail the block treatment already noted.

The number of grains per earhead and the thousand-corn weight of the grain were determined at harvest for the wheat on Long Hoos: no effect of cultivation or fertilizer was found.

The analysis of the yield of barley, given in Table VII, shows that the yield per plant tends to be highest on the grubbed plots, which have the lowest plant density, and lowest on the rototilled plots with the highest plant density. This is the normal relation between yield per plant and plant density. The yield per shoot also tends to be highest on the grubbed and lowest on the ploughed plots, which is in the inverse order of the shoot density.

Table VII. *Analysis of barley yield. Long Hoos*, 1934

| | Plough | Roto-tiller | Grubber | Mean | Plough | Roto-tiller | Grubber | Mean |
|---|---|---|---|---|---|---|---|---|
| | Plants per metre at harvest (approx.) | | | | Ear-bearing shoots per metre at harvest | | | |
| Deep | 36·2 | 37·2 | 28·7 | 34·0 | 66·0 | 65·2 | 57·6 | 62·9 |
| Shallow | 31·6 | 32·9 | 32·7 | 32·4 | 61·8 | 53·2 | 54·9 | 56·7 |
| Mean | 33·9 | 35·0 | 30·7 | — | 63·9 | 59·2 | 56·2 | — |
| | Yield of grain, grams/metre | | | | Yield of straw, grams/metre | | | |
| Deep | 62·6 | 66·7 | 64·2 | 64·5 | 64·5 | 65·9 | 64·6 | 65·0 |
| Shallow | 64·0 | 58·0 | 62·5 | 61·5 | 65·9 | 61·4 | 60·8 | 62·7 |
| Mean | 63·3 | 62·3 | 63·4 | — | 65·2 | 63·6 | 62·7 | — |
| | Yield of grain, grams/plant | | | | Yield of grain, grams/shoot | | | |
| Deep | 1·73 | 1·79 | 2·24 | 1·92 | 0·95 | 1·02 | 1·11 | 1·03 |
| Shallow | 2·02 | 1·76 | 1·91 | 1·90 | 1·03 | 1·09 | 1·14 | 1·09 |
| Mean | 1·88 | 1·77 | 2·08 | — | 0·99 | 1·05 | 1·13 | — |
| | Yield of grain, grams/metre computed from shoot number, grains/earhead, and thousand-corn weight | | | | | | | |
| Deep | 63·9 | 68·1 | 64·3 | 65·4 | | | | |
| Shallow | 66·0 | 59·2 | 58·5 | 61·2 | | | | |
| Mean | 65·0 | 63·6 | 61·4 | — | | | | |

As with wheat, the thousand-corn weight and the number of grains per earhead were both determined, and though both vary somewhat with different treatments, the differences are not significant. They do, however, enable the yield to be calculated from them using the number of shoots bearing ears. The agreement between this computed yield and the actual yield is good except for the shallow-grubbed whic<sup></sup> yield better than expected. The low yield of the shallow-rototilled plots is thus probably a consequence of low shoot number.

The detailed effects of plant number, shoot number and the number of grains per earhead on the grain and straw yield, when the main soil and cultivation treatments have been allowed for, were negligible in contrast with the sensible effect of shoot number on grain yield of wheat.

*The growth of the mangold crop. Long Hoos*, 1934

The number of plants at harvest was not dependent on the number that had germinated, since sufficient had germinated to allow a fairly uniform plant to be set at singling. Thus although germination was much better on the ploughed plots than on the grubbed and rototilled plots, this effect was lost after singling, as is shown in Table VIII.

Table VIII. *Plant number at harvest, in thousands/acre*

| | Plough | Rototiller | Grubber | Mean (1) |
|---|---|---|---|---|
| Deep tilled | 21·4 | 21·5 | 21·8 | 21·6 |
| Shallow tilled | 22·4 | 21·2 | 20·6 | 21·4 |
| Mean (2) | 21·9 | 21·4 | 21·2 | — |

Standard errors of mean (1) = 0·26, of mean (2) = 0·31.

The number of leaves per plant was determined seven times at fortnightly intervals up to harvest. No appreciable difference in the rate of increase of leaf number due to cultivation was found. The number of leaves per root at harvest was:

| | Plough | Rototiller | Grubber | Mean (1) |
|---|---|---|---|---|
| Deep tilled | 42·5 | 42·7 | 45·1 | 43·5 |
| Shallow tilled | 45·5 | 44·4 | 45·9 | 45·2 |
| Mean (2) | 43·9 | 43·6 | 45·5 | — |

Standard errors of mean (1) = 0·64, of mean (2) = 0·79.

showing that it tends to be higher on the shallow than on the deep-tilled plots.

The circumference of the roots at ground level was determined five times at fortnightly intervals. The mangolds on the shallow-tilled plots had consistently larger roots at all five sampling dates than those on the deep-tilled plots, and this difference was statistically significant at each

Table IX. *Circumference of mangold roots at harvest in cm.*

| | Plough | Rototiller | Grubber | Mean (1) |
|---|---|---|---|---|
| Deep tillage | 43·5 | 44·9 | 45·8 | 44·7 |
| Shallow tillage | 45·4 | 46·1 | 48·9 | 46·8 |
| Mean (2) | 44·4 | 45·5 | 47·3 | — |

Standard errors of mean (1) = 0·71, of mean (2) = 0·87.

date. The mangolds on the grubbed plots were larger than those on the ploughed or rototilled plots at all five sampling dates, though these differences did not achieve significance. The mean circumferences at harvest are given in Table IX.

*Analysis of yield of the mangold crop*

The yields and means used in this section are calculated from the yields of the whole plots, and not, as in the preceding paper, from the yields of each plot when the two outside rows are rejected, i.e. from the two central rows only. The effect of the cultivation treatments on the yield of the mangolds is given in Table X.

Table X. *Effect of the seed-bed preparation on the yield of mangolds. (On the whole plot basis, no rows rejected)*

Yield of mangolds in tons/acre

| | Roots | | | | Tops | | | |
|---|---|---|---|---|---|---|---|---|
| | Plough | Rototiller | Grubber | Mean | Plough | Rototiller | Grubber | Mean |
| Deep | 40·4 | 37·4 | 39·9 | 39·6 | 8·33 | 7·75 | 7·94 | 8·00 |
| Shallow | 40·3 | 37·1 | 36·7 | 38·0 | 8·16 | 8·53 | 8·20 | 8·30 |
| Mean | 40·4 | 37·2 | 38·3 | — | 8·25 | 8·14 | 8·07 | — |

Standard error for each treatment 0·98 tons/acre for roots and 0·25 tons/acre for tops.

| | Mean weight per root (in lb.) | | | | | Mean green weight of leaves per plant (in lb.) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Plough | Roto-tiller | Grubber | Mean (1) | | Plough | Roto-tiller | Grubber | Mean (3) |
| Deep | 4·25 | 3·90 | 4·06 | 4·07 | | 0·877 | 0·810 | 0·820 | 0·836 |
| Shallow | 4·05 | 3·91 | 4·04 | 4·00 | | 0·822 | 0·901 | 0·905 | 0·876 |
| Mean (2) | 4·15 | 3·91 | 4·05 | — | Mean (4) | 0·850 | 0·855 | 0·863 | — |

Standard error of means: (1) 0·0648; (2) 0·0795; (3) 0·0163; (4) 0·0199.

| | Mean green weight per leaf (in lb.) | | |
|---|---|---|---|
| | Plough | Rototiller | Grubber |
| Deep | 0·0206 | 0·0189 | 0·0181 |
| Shallow | 0·0181 | 0·0203 | 0·0197 |

The deep-ploughed plots have the highest yield per acre and per root and the rototilled plots the lowest, while, as shown in Table VIII, the plant number is practically the same. The depth of tillage on the rototilled and grubbed plots has no effect on the weight of the root, but the roots are lighter on the shallow than on the deep-ploughed plots. Comparing Table X with the preceding Table IX, on the circumference of mangold roots at harvest, the deep-ploughed plots give both the heaviest root and that with the smallest circumference, i.e. the thinnest,

*longest, but heaviest roots.* On the other hand, the rototilled and grubbed plots give lighter roots with a larger circumference, i.e. squatter roots. Further, the shallow-tilled plots tend to give squatter roots than the deep-tilled for each of the three types of cultivation.

The deep-ploughed, the shallow-rototilled and the shallow-grubbed plots give mangolds having heavier tops per plant than the other three treatments. This is not because they have more leaves per plant, but because, as shown in the bottom of Table X, they have heavier individual leaves.

The effect of the plant numbers, number of leaves per plant, and the circumference of the root on each other and on the yield of roots and tops was investigated. The circumference of the roots was influenced by the number of leaves, and the yield of roots and tops tended to increase as the plant number and root circumference increased but not as the number of leaves per plant increased.

The effect of spacing could be determined directly since the plots were harvested in three sections. There are four mangold rows per plot. The centre two rows have a 22 in. spacing and each of the outer two rows have, because of the paths between the plots, the unsymmetrical spacing of 22 in. on one side and 38 in. on the other. Table XI shows the increase of yield of the outer rows compared with the inner, and is given in lb. per row in the left-hand half of the table and as a percentage increase over the inner rows in the right-hand half.

Table XI. *Increase of yield of wider spaced outer rows over inner rows*

| | Actual increase of weight in lb. per row (of 139·8 links or 28·1 metres) | | | | Percentage increase in weight of outer rows compared with inner rows | | | |
|---|---|---|---|---|---|---|---|---|
| | Plough | Rototiller | Grubber | Mean | Plough | Rototiller | Grubber | Mean |
| Deep | 44·8 | 54·8 | 30·2 | 43·3 | 13·7 | 18·5 | 9·3 | 13·8 |
| Shallow | 59·2 | 46·1 | 3·3 | 36·2 | 18·7 | 15·5 | 1·1 | 11·8 |
| Mean | 52·0 | 50·5 | 16·7 | — | 16·2 | 17·0 | 5·2 | — |

The mangolds growing on the shallow-grubbed plots obviously had all the space they needed in a 22 in. row, while those growing on the ploughed or rototilled plots could utilize the extra space. The poor response of the mangolds on the grubbed plots is reflected in the increased response of the mangolds on an adjacent plot. For the increase of yield of an outside row of a plot when the adjacent plot was a ploughed plot, a rototilled plot or a grubbed plot was 41, 37 and 56 lb. per row respectively.

*Summary of the effects of cultivation treatments on the growth of the mangold crop*

Germination can be reduced if there are many weeds competing with the mangolds, but although many more seeds germinated on the relatively clean ploughed plots, there were enough on the rototilled and grubbed plots to allow an even plant after singling. The shape of the roots is affected by the depth of cultivation, being squatter on the shallow and longer on the deep-worked plots. The roots appeared to be smaller on the rototilled plots than on the ploughed or grubbed plots, but there was no reserve of strength in the plants on the grubbed plots, for if the spacing between the rows was increased they could not utilize this extra space, while the plants on the ploughed and rototilled plots could do so.

## V. EFFECT OF CULTIVATION ON THE GROWTH OF WEEDS

The object of this section is to examine how great an increase of weed population occurs on plots worked with the Rototiller or the grubber compared with those that are ploughed. It would be an important limitation, though not a fatal objection to the employment of these implements in place of the plough if weeds accumulated too badly under them. Two separate problems thus arise, namely how badly do the weeds accumulate from year to year and by how much do they depress the crop yield in any particular year. From investigations such as these it should be possible to estimate how seriously a given weed population does reduce the crop yield, a point of great economic importance in mechanized crop husbandry.

The effect of different methods of preparing the seed bed on the weed population during the subsequent growing season has been investigated by four methods, namely:

(1) Eye estimate of weediness.

(2) Laying down a 12 in. square wire screen divided into a hundred squares and counting the number of squares in which a weed occurs.

(3) Counting the number of weed seeds in the soil by the Brenchley & Warington (1930) method after the crop has been harvested.

(4) Determining the number and the dry matter of the weeds per square metre.

The weediness of plots under different cultivation treatments is naturally very dependent on the weather. In most years for the cereal crops there was no difference visible to the eye on plots receiving

different cultivations, though eye estimates of weediness made on the Little Hoos wheat plots on 7 April 1931 showed that the eight rototilled plots were all in the "very weedy" class, the pulverator-ploughed plots were mainly in the "fairly weedy" class, while the ploughed plots were in the "very few weeds" class.

The mangolds plots on the Long Hoos cultivation experiment, however, do show up differences in weediness that are easily visible to the eye. Both in 1934 and in 1935 the ploughed plots were definitely the least weedy and the grubbed the most weedy with the rototilled plots intermediate. Also the plots which were only worked shallow with either of these implements were weedier than those worked deep. In fact the problem has become so serious that now the wheat stubble is shallow ploughed in autumn in preparation for the mangold break.

The number of weeds per square metre was determined on the Long Hoos wheat plots seven times at fortnightly intervals from 12 May to 3 August 1934, and on the barley plots twice on 19 July and 2 August 1934. The plots on each of these experiments are recorded as being fairly free from weeds in the harvest notes taken at the time.

On the Long Hoos wheat there were on the average 127 weeds per square metre on 12 May. On 22 May the plots were hand-hoed, but 4 days later they appear to have had 111 weeds per square metre, and a fortnight later a note occurs that flowering weeds were pulled out, so that this hand-hoeing must have had little or no effect. At each of the seven counts the ploughed plots had significantly fewer weeds than those rototilled or grubbed; there was no difference between the rototilled and grubbed plots nor between the deep and shallow tilled. The mean number of weeds per square metre for the seven counts were:

| | Plough | Rototiller | Grubber | Mean |
|---|---|---|---|---|
| Deep | 84 | 96 | 96 | 92 |
| Shallow | 80 | 93 | 93 | 89 |
| Mean | 82 | 95 | 95 | — |

The number of weeds per square metre had no influence on the plant number, shoot number, height of shoot, yield of grain or yield of straw for the wheat, presumably because there were not sufficient weeds present to interfere seriously with the wheat crop.

The mean number of weeds per square metre on the barley plots between 19 July and 2 August 1934 were:

| | Plough | Rototiller | Grubber | Mean |
|---|---|---|---|---|
| Deep | 100 | 87 | 85 | 90 |
| Shallow | 94 | 97 | 78 | 90 |
| Mean | 97 | 92 | 81 | — |

The differences between treatments are barely significant. This is the only result yet obtained where the ploughed plots had most weeds and the grubbed plots least. But again the field was recorded as being fairly clean and there were not enough weeds to affect the growth of the barley crop.

The weed problem was followed up in great detail in the experiment on Pastures Field in 1932–3 (Keen, 1933). The field was in wheat and the comparisons were between ploughing and rototilling when they were carried out either as deep or as shallow tillage; on this was superimposed one between no spring cultivation, rolling alone, harrowing alone and rolling and harrowing together; and on this was further superimposed a comparison between a top dressing of sulphate of ammonia and no top dressing. A plan of this experiment has been given by Keen (1933). In September 1933, after the wheat had been carted off the field, a survey was made of the weediness of the different stubbles by using the wire grid. The results expressed as the average percentage of squares containing weeds were as follows:

| Plough | Rototiller | Deep tilled | Shallow tilled | No top dressing |
|---|---|---|---|---|
| 25·7 | 34·6 | 28·8 | 31·3 | 28·2 |

| Top-dressed with sulphate of ammonia | No spring cultivation | Rolled only | Harrowed only | Rolled and harrowed |
|---|---|---|---|---|
| 31·9 | 32·7 | 31·1 | 32·2 | 24·2 |

The statistical tests of significance showed the difference between the plough and the Rototiller, between no top dressing and sulphate of ammonia and between rolling and harrowing together and no spring cultivation were significant while the others were not.

Just before the land was ploughed samples were taken so that an estimate of the weed-seed content of the plots could be determined. The effects of spring cultivation were ignored so that the effects of only eight treatments, i.e. all combinations of plough *v.* Rototiller, deep *v.* shallow tillage, nitrogenous top dressing *v.* none were examined. The lay-out of the experiments (Keen, 1933) was that forty-eight main plots were divided into six blocks running east and west in two parallel lines of three blocks each. One line of blocks will for convenience be referred to as the south line and one as the north line. Each main plot is divided into two, one half having received a top dressing of sulphate of ammonia in the spring. Each of these eight treatments occurs twice ir each block, that is six times in each line. The duplicates in each block were now assigned the number 1 or 2 at random and two samples from each plot

were taken. To reduce the number of samples they were mixed in the following way. For each line and each treatment the two samples from each of the three plots numbered 1 were mixed giving a bulk sample *composed of six separate samples. Similarly* a duplicate sample for the *same line and same treatment were made by mixing the six samples from* the plots numbered 2. Hence there were four replicates for each of the eight treatments, two from each of the two lines. Each bulked sample contained 288 cu. in. of soil, for the depth of the sample was 2 in. and the area of each individual sample 24 sq. in. Samples were taken at four depths: 0–2, 2–4, 4–6, 6–8 in. on the deep-tilled plots and from 0–2 and 2–4 in. from the shallow-tilled plots. The bulk of the samples was reduced by washing in a stream of water on a 100-mesh sieve, which is fine enough to retain all weed seeds but which allows much of the fine sand, silt, and clay to be washed away. These samples, to which some coarse sand may be added, were then put in earthenware pans and kept in a glasshouse to allow the weed seeds to germinate. The pans were under observation from October 1933 to March 1937 and the weeds that had germinated were identified, counted, and picked out at regular intervals.

The number of all weed seeds per acre in the top 2 in. of soil under the different cultivation treatments is given in Table XII.

Table XII. *Number of weed seeds in top 2 in. of soil in millions per acre*

| | Plough | | | Rototiller | | | Mean | | |
|---|---|---|---|---|---|---|---|---|---|
| | Deep | Shallow | Mean | Deep | Shallow | Mean | Deep | Shallow | Mean |
| South line | 31·4 | 50·9 | 41·2 | 40·1 | 53·4 | 46·7 | 35·8 | 52·3 | 44·0 |
| North line | 14·2 | 17·6 | 15·9 | 28·6 | 17·9 | 23·2 | 21·4 | 17·8 | 19·6 |
| Mean | 22·8 | 34·3 | 28·6 | 34·3 | 35·6 | 34·9 | 28·6 | 35·0 | 31·8 |

The table shows that there is a very large difference between the two lines, one having more than twice as many weed seeds as the other. The deep ploughed has consistently the smallest number of weed seeds and there is very little difference between the shallow-ploughed and the shallow-rototilled plots. But the deep-rototilled plots do not give any consistent result. Each entry in this table is the mean of the counts on four independent samples. In the north line the four highest counts were the four deep-rototilled plots, while in the south line two of the deep-rototilled samples had much smaller counts than the corresponding shallow-rototilled, one had about the same and one rather more. Hence the inconsistency of the deep-rototilled plots is not due to an abnormal

number of seeds in just one of the samples. The effects of the different treatments can be seen in another way from Table XIII.

Table XIII. *Percentage increase in the number of weed seeds in the top 2 in. of soil due to certain treatments*

| | Rototiller—Plough | | | Top dressing—None | | | Deep—shallow tillage |
|---|---|---|---|---|---|---|---|
| | Deep | Shallow | Mean | Deep | Shallow | Mean | |
| South line | 21·2 | 4·5 | 12·8 | 30·4 | 47·0 | 38·7 | 24·6 |
| North line | 67·6 | 1·4 | 34·5 | 9·4 | −3·2 | 3·1 | −18·8 |
| Mean | 44·4 | 3·0 | 23·7 | 19·9 | 21·9 | 20·9 | 2·9 |

This table shows that both the nitrogen top dressing and shallow tillage increase the number of weed seeds in one line while in the other the top dressing has no effect and shallow tillage reduces the number of weed seeds.

Table XIV shows that very nearly the same results are obtained if the number of seeds of a specific weed is used instead of grouping all weed seeds together. The commonest weeds were *Alopecurus agrestis*, *Alchemilla arvensis*, *Polygonum arviculare* and miscellaneous grasses, then came *Stellaria media*, *Matricaria inodora* and *Juncus bufonius*. All other

Table XIV. *Distribution of weed seed species. Top 2 in. before cultivation.* (*Total numbers in samples*)

| | Deep ploughed | Shallow ploughed | Deep roto-tilled | Shallow roto-tilled | Deep | Shallow | Ploughed | Roto-tilled | Total |
|---|---|---|---|---|---|---|---|---|---|
| *Alopecurus agrestis*: | | | | | | | | | |
| South | 731 | 1002 | 961 | 1280 | 1692 | 2282 | 1733 | 2241 | 3974 |
| North | 527 | 462 | 943 | 436 | 1470 | 898 | 989 | 1379 | 2368 |
| *Alchemilla arvensis*: | | | | | | | | | |
| South | 848 | 1153 | 631 | 813 | 1479 | 1966 | 2001 | 1444 | 3445 |
| North | 144 | 266 | 354 | 269 | 498 | 535 | 410 | 623 | 1033 |
| *Stellaria media*: | | | | | | | | | |
| South | 88 | 143 | 141 | 187 | 229 | 330 | 231 | 328 | 559 |
| North | 66 | 168 | 247 | 297 | 313 | 465 | 234 | 544 | 778 |
| *Polygonum aviculare*: | | | | | | | | | |
| South | 738 | 975 | 624 | 704 | 1362 | 1679 | 1713 | 1328 | 3041 |
| North | 265 | 466 | 603 | 297 | 868 | 763 | 731 | 900 | 1631 |
| Grasses spp. | | | | | | | | | |
| South | 43 | 336 | 856 | 1049 | 899 | 1385 | 379 | 1905 | 2284 |
| North | 34 | 55 | 186 | 130 | 220 | 185 | 89 | 316 | 405 |
| *Matricaria inodora*: | | | | | | | | | |
| South | 226 | 468 | 138 | 295 | 364 | 763 | 694 | 433 | 1127 |
| North | 59 | 105 | 159 | 88 | 218 | 193 | 164 | 347 | 411 |
| *Juncus bufonius*: | | | | | | | | | |
| South | 165 | 393 | 114 | 114 | 279 | 507 | 558 | 228 | 786 |
| North | 3 | 4 | 11 | 3 | 14 | 7 | 7 | 14 | 21 |

species were much less common. In every case the south side had more seeds than the north and the rototilled north plots had more than the ploughed north plots. On the south plots the weed seeds of some of the common species were more numerous on the ploughed and of others on the rototilled plots. There were no consistent differences between deep and shallow tillage.

The effect of tillage on weed seeds below the surface 2 in. can be examined in two parts. The method of sampling used was that only the depths 0–2 and 2–4 in. were sampled on the shallow-tilled plots, while in addition the depths 4–6 and 6–8 in. were sampled on the deep-tilled plots. The first comparison then in which all the eight treatments occur is a comparison of the weed seeds in the subsurface layer 2–4 in. Table XV gives the difference between the weed seeds in two treatments as a percentage of the mean number of weed seeds present.

Table XV. *Percentage increase in the number of weed seeds in the subsurface layer (2–4 in.) due to certain treatments*

| | Rototiller—Plough | | | Top dressing—none | | | Shallow—deep |
|---|---|---|---|---|---|---|---|
| | Deep | Shallow | Mean | Deep | Shallow | Mean | |
| South line | 1·3 | −15·5 | −7·1 | 1·8 | 47·2 | 24·5 | 11·8 |
| North line | 48·2 | 6·5 | 27·3 | 19·3 | 7·6 | 13·5 | 7·0 |
| Mean | 24·7 | −4·5 | 10·2 | 10·5 | 27·4 | 19·0 | 9·4 |

The only conclusions that can be drawn are that the top dressing has probably increased and deep tillage probably decreased the number of weed seeds. The comparison of the ploughed and the rototilled are too erratic to give any conclusion.

The effect of tillage on the distribution of weed seeds between 2–8 in. in depth on the deep-tilled plots is given in Table XVI.

Table XVI. *Percentage increase in the number of weed seeds due to certain treatments*

| | Rototiller—plough | | | Top dressing—none | | |
|---|---|---|---|---|---|---|
| Line of blocks | South | North | Mean | South | North | Mean |
| Depth (in.) | | | | | | |
| 2–4 | 1·3 | 48·2 | 24·8 | 1·9 | 19·3 | 10·6 |
| 4–6 | 18·6 | 32·2 | 25·4 | −21·7 | 10·8 | −5·5 |
| 6–8 | −21·4 | −18·4 | −19·7 | 11·5 | −23·5 | −6·0 |

Thus the rototilled plots seem to have more weed seeds than the ploughed down to a depth of 6 in., and for the last 2 in. the ploughed have more weed seeds than the rototilled. This is probably the result

of the previous ploughing, for after these plots had been cultivated they were sampled again, and percentage excess of weed seeds on the rototilled plots over the ploughed plots were then:

| Depth (in.) | South line | North line | Mean |
|---|---|---|---|
| 2–4 | 17·4 | 30·8 | 24·1 |
| 4–6 | −15·2 | −33·7 | −24·5 |
| 6–8 | −39·5 | −37·8 | −38·7 |

showing that the ploughed plots had a large excess below 4 in. depth. The apparent effect of the top dressing below the 4 in. depth, given in Table XVI, is probably entirely due to sampling errors.

Thus the main conclusions reached from this examination are that differences in cultivation treatment do not appear to encourage certain species of weeds in preference to others and that the number of weed seeds in the surface layer was lowest on the deep ploughed plots, and the number in the 6–8 in. layer was highest on these plots; this latter effect being due to the deep burial of the surface weed seeds by the ploughing, many of which can retain their viability for 12 months. There were no other consistent effects except that there was very little difference between the shallow-plough and shallow-rototiller plots.

*Dry matter of the weeds and wheat on Pastures* 1933–4

During the growing season of 1934 the number and dry matter of both weed and wheat plants were determined five times at approximately monthly intervals beginning on 5 April and ending 26 July by harvesting the wheat and weeds separately on two areas of 0·152 sq. m. per plot. In the top half of Table XVII is given the number of weed seeds per square metre found in the top 2 in. and the top 4 in. of soil after cultivation in November 1933, and in the bottom half the number of weeds counted during the growing season of 1934. There is a general correlation between the number of weed seeds present in the surface soil and the number of weeds present from May onwards, as can be seen by comparing the differences shallow—deep which run in the same direction in the two parts of the table. The difference rototiller—plough runs in the same order if the weed seeds in the first 4 in. are compared with the weeds present in the beginning of April, or the weed seeds in the first 2 in. and the weeds present in May. There are consistent effects shown by the differences between the plots top-dressed in April 1933 and those not top-dressed. A further top dressing of sulphate of ammonia was given on 10 April 1934 to those plots which were top-dressed in 1933.

Table XVII

*Weed seeds present in the surface soil in November* 1933 *in thousands per square metre*

| | Ploughed plots | | Rototilled plots | | Roto-tiller—plough | Top-dressed—none |
|---|---|---|---|---|---|---|
| | *Top-dressed* | *Not top-dressed* | *Top-dressed* | *Not top-dressed* | | |
| | In the top 2 in. of soil | | | | | |
| Deep tillage | 2·21 | 2·68 | 7·25 | 3·30 | 2·83 | 1·74 |
| Shallow tillage | 6·83 | 3·78 | 6·70 | 5·31 | 0·70 | 2·22 |
| Shallow—deep tillage | 4·62 | 1·10 | −0·55 | 2·01 | — | — |
| | In the top 4 in. of soil | | | | | |
| Deep tillage | 5·51 | 6·52 | 12·58 | 6·92 | 3·73 | 2·83 |
| Shallow tillage | 17·42 | 10·30 | 12·43 | 11·68 | −1·80 | 3·93 |
| Shallow—deep tillage | 11·91 | 3·78 | −0·15 | 4·76 | — | — |

*Number of weeds per square metre in* 1934

| | Ploughed plots | | Rototilled plots | | Roto-tiller—plough | Top-dressed—none |
|---|---|---|---|---|---|---|
| | Top-dressed | Not top-dressed | Top-dressed | Not top-dressed | | |
| | Counted on 5. iv. 34 | | | | | |
| Deep tillage | 105 | 142 | 165 | 220 | 69 | −46 |
| Shallow tillage | 159 | 232 | 241 | 172 | −30 | −2 |
| Shallow—deep tillage | 54 | 90 | 76 | −48 | — | — |
| | Mean of counts made on 3. v. 34 and 31. v. 34 | | | | | |
| Deep tillage | 168 | 280 | 646 | 416 | 307 | 59 |
| Shallow tillage | 396 | 438 | 573 | 570 | 154 | −20 |
| Shallow—deep tillage | 228 | 158 | −73 | 154 | — | — |
| | Mean of counts made on 28. vi. 34 and 26. vii. 34 | | | | | |
| Deep tillage | 164 | 218 | 500 | 392 | 619 | 163 |
| Shallow tillage | 318 | 268 | 420 | 462 | 396 | 8 |
| Shallow—deep tillage | 154 | 50 | −80 | 70 | — | — |

The effect of cultivations on the dry matter of the weeds at different dates is given in Table XVIII. The general results are that the dry matter of the weeds is higher on the rototilled than on the ploughed and higher on the shallow-tilled than on the deep, while exactly the reverse is true for the dry matter of the wheat. The extra dry matter of the weeds on the rototilled plots and of the wheat on all the deep-tilled plots are statistically significant ($P < 00{\cdot}1$) at all five counts. The dry matter of the wheat on the deep-ploughed and the deep-rototilled plots is practically the same, but the dry matter on the shallow-rototilled plots is definitely less than on the shallow-ploughed. With the weeds, the dry matter is always least on the deep ploughed, greatest on the shallow rototilled, and approximately the same on the deep rototilled and the shallow ploughed. Both the weeds and the wheat tended to have a

higher dry matter on the plots top-dressed with sulphate of ammonia than those not top-dressed, but the increases were only statistically significant for the weeds at the third and fourth counts.

Table XVIII.

*Effect of cultivation on the dry matter of the weeds (in grams per square metre)*

| Date of sampling | Mean dry matter g./sq. m. | 1932 cultivation Rototiller —plough | 1933 cultivation Rototiller —plough | Shallow —deep | Standard error | Sulphate of ammonia —none | Standard error |
|---|---|---|---|---|---|---|---|
| 5. iv. 34 | 1·72 | 0·09 | 0·83 | − 0·72 | 0·24 | −0·41 | 0·18 |
| 3. v. 34 | 9·00 | −0·13 | 7·92 | 3·74 | 1·18 | 1·64 | 0·79 |
| 31. v. 34 | 75·4 | −5·51 | 30·5 | 11·2 | 4·20 | 27·0 | 4·53 |
| 28. vi. 34 | 95·6 | −3·22 | 39·6 | 38·2 | 6·30 | 20·8 | 6·24 |
| 26. vii. 34 | 132·1 | +5·51 | 51·4 | 24·6 | 10·0 | 20·9 | 10·50 |

*Effect of cultivation on the dry matter of the wheat (in grams per square metre)*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 5. iv. 34 | 3·82 | 0·09 | − 1·47 | − 1·13 | 0·32 | 0·24 | 0·31 |
| 3. v. 34 | 12·1 | − 2·35 | − 1·51 | − 4·46 | 0·79 | −0·26 | 0·85 |
| 31. v. 34 | 104·5 | − 3·68 | − 9·0 | −22·5 | 6·30 | 11·6 | 6·76 |
| 28. vi. 34 | 242·5 | − 7·9 | 11·8 | −59·0 | 12·9 | 20·6 | 11·4 |
| 26. vii. 34 | 432·0 | −12·1 | 3·48 | −120·2 | 24·0 | 24·2 | 21·2 |
| Harvest: | | | | | | | |
| Grain | 137·2 | − 3·3 | −12·4 | −32·2 | 10·3 | −2·8 | 6·08 |
| Straw | 170·0 | − 4·9 | −28·1 | −39·2 | 9·97 | −6·2 | 7·07 |
| Total | 307·2 | − 8·2 | −40·5 | −71·4 | — | −9·0 | — |

A further point was studied in the weed count made in May. The number of perennial weeds were counted on 7 May 1934 on each plot at a time when the annuals were small, and the results, expressed as the number of perennials per acre were:

| Treatment | Ploughed: Deep | Ploughed: Shallow | Rototilled: Deep | Rototilled: Shallow |
|---|---|---|---|---|
| Top-dressed with S.A. April 1933 | 993 | 4193 | 2707 | 4160 |
| Not top-dressed | 1529 | 4067 | 2840 | 6320 |

Clearly the deep-ploughed plots have the fewest perennials and the shallow-tilled plots the most. The shallow-rototilled plots that were not top-dressed with nitrogen the previous year possess more perennials than those top-dressed. The cultivation given the previous year, i.e. the ploughing or rototilling given in the autumn of 1932, has no effect on this result, which, as a matter of fact, is not easy to understand, for one would not have expected such a large number of perennials on the shallow ploughed compared with the deep, nor that the top dressing would have depressed the number of perennials on the deep-ploughed and shallow-rototilled plots.

The effect of the weeds on the growth of wheat was examined by computing the regression of the dry matter of the weeds on the dry matter of the wheat using three sets of data, namely, the comparison of plot yields, of subplot yields, and of the two samples taken per subplot. For the first three sampling times, namely in April and May, the weeds and the wheat appeared to grow independently of each other, but in June and July all three comparisons showed that where the growth of weeds was strong the growth of wheat was poor. Unfortunately from the data it is not possible to say if the wheat was poor because the weeds grew strongly or *vice versa*. If it was due to the former cause these results cannot be applied directly to a normal wheat crop, because, as already mentioned, this had only half the number of plants that a normal one would have, and the individual plants were poorer than those on a good wheat field before the weeds started to grow. It is very probable in fact that the weeds were strong because the wheat was poor and thin.

The data collected allow one to examine a secondary question, namely the effect of the top dressing of nitrogen on the growth of weeds and wheat. Its effect on the wheat has been small, although at the end of May the dry matter per wheat plant was 10 % higher on the top-dressed ploughed plots than on the undressed ones and 20 % higher on the top-dressed rototilled plots than on the undressed ones. At the end of July these differences had sunk to 6·2 and 3·5 % respectively, and at harvest the nitrogen top dressing increased the yield of grain and straw by 2·2 % on the ploughed and depressed it by 21·5 % on the shallow-rototilled plots. The effect of the nitrogen on the weeds was to increase the dry matter per weed by 15 and 46 % on the ploughed and rototilled plots at the end of May, but by the end of July it had no effect on the weeds on the ploughed plots and caused an increase of 19 % on the rototilled plots. A further result was that the wheat was only able to compete successfully with the weeds for the nitrogen added in the top dressing on the deep-tilled plots, as shown in Table XIX.

Table XIX. *The yield of nitrogen in the wheat and in the weeds, in grams per square metre. Sampled on* 31 *May* 1934

| | Wheat | | Weeds | |
|---|---|---|---|---|
| Tillage | Deep | Shallow | Deep | Shallow |
| Top dressing: | | | | |
| Present (N) | 2·28 | 1·87 | 1·25 | 1·91 |
| Absent (O) | 1·84 | 1·90 | 1·16 | 1·13 |
| N—O | 0·44 | −0·03 | 0·09 | 0·78 |

Nitrogen added in top dressing = 2·5 g./sq. m.

In spite of this difference in the yield of nitrogen, the nitrogen content of the weeds was highest on the deep-tilled top-dressed plots. As the season progressed the effect of the top dressing on the yield of nitrogen decreased both in the wheat and in the weeds. Hence the nitrogenous top dressing, put on this field which carried a very poor wheat crop, appears to have benefited the weeds more than the wheat, particularly on those plots which tended to be weedy in any case.

The main points that have come out from this section are that the rototilled and grubbed plots tend to be weedier than plots that have been ploughed, due in part to the inability of these implements to bury the more troublesome perennial weeds and in part to their leaving more weed seeds in the surface layer. This effect is usually unimportant when these implements have been used for one year only but tends to become much more troublesome if they are used for several years in succession or if the crop is poor for any extraneous reason.

## SUMMARY AND CONCLUSIONS

The main conclusions that can be drawn from these field experiments to compare the effect of different methods of preparing the seed bed on the crop's growth are:

(1) Crops germinate faster on the looser seed bed prepared by a Rototiller than on the more compact ones prepared by a plough or a grubber. The total number of plants that germinate is, however, the same for all treatments unless the land is too foul with weeds, when higher germination is obtained on the cleaner plots.

(2) Cereals tend to ripen a little sooner on land that has been ploughed than on land that has been either rototilled or grubbed, but in most years this effect if present is very small.

(3) The shape and the weight of the mangold root seems to depend on the seed bed. The roots were longest and thinnest on the deep-ploughed plots and were always squatter on the shallow-tilled than on the deep-tilled plots. The roots were heaviest on the deep-ploughed plots and lightest on the rototilled plots. On the rototilled and the grubbed plots the depth of tillage had no effect. The plants on the shallow-grubbed plots seemed, however, to have no reserve of strength, for they could not make better growth if given more room, while those on the deep-grubbed plots could make some use and those on the ploughed or rototilled plots appreciable use of extra space.

(4) Weeds tend to accumulate on the rototilled and the grubbed plots

since neither grubbers nor rotary cultivators carrying tines mounted on a horizontal shaft can bury weeds and weed seeds in the way that the plough can. If the land is fairly clean and in good heart this probably does not matter for several years, but it prevents either of these from completely displacing the plough.

(5) *A subsidiary result that emerged from these experiments is that if a thin crop is given a nitrogenous top dressing, the fertilizer may* benefit the weeds more than the crop.

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# FIELD EXPERIMENTS OF FACTORIAL DESIGN

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A RECENT paper by A. E. Brandt (1937) goes into the details of a type of design in field experimental procedure, where two or more factors are under examination, which has been much elaborated since 1928, when the first $2 \times 2 \times 2$ experiment, involving two levels of each of three factors, nitrogen, potash and phosphate, was carried out at Woburn (*Rothamst. Rep.* 1927–8) with four-fold replication. Similar experiments, of the $3 \times 2 \times 2$ type, had in fact been conducted at Rothamsted (*Rep.* 1925–6) two years earlier, but there was here the further complication that no differentiation was possible for two of the factors at one level (no manure) of the third. Details have been given by Fisher (1937) and Yates (1937) of, among others, experiments of the $2^n$ and $3 \times 2^n$ types, and it may be said that the recent work has been in the direction of systematizing the lay-out and analysis of such experiments. Further features have been the device of confounding, which dates back to 1927 (*Rothamst. Rep.* 1927–8), i.e. it is almost contemporaneous with the first introduction by Fisher of randomized blocks and Latin square experiments, and the suggestion that replication may even be dispensed with entirely, a much more recent innovation. Confounding is a method of enlarging the number of blocks between which elimination of soil heterogeneity is possible by sacrificing information on certain of the higher-order interactions, which are considered unlikely to be real effects; with absence of replication an estimate of the experimental error is found by grouping together a number of these higher-order interactions.

Since the introduction of such experiments, it has always been recognized that, to illustrate from the $2 \times 2 \times 2$ type, the significance of the main effects and interactions can be examined by splitting up the 7 degrees of freedom for treatments into the 7 single degrees of freedom for the various comparisons possible between two groups of four treatments each. Since then there has been a tendency to extend this process. Thus, in Brandt's first experiment the two degrees of freedom for the three lengths of time were split up into two single degrees of freedom, and this applied also to all the interactions with the other factors. This is, of course, only a logical application of Fisher's (1937) suggestion for dealing with a case of three levels of an ingredient. It means, however,

that we must frequently face the situation, as in Brandt's case, where not only are the treatment mean squares based on only one degree of freedom each, but also the error, in the absence of replication, is a composite of a number of single degrees of freedom.

The purpose of this paper is to direct attention to the examination for significance of treatment effects based upon single degrees of freedom. It is an elementary statistical point that a mean square, based on 1 degree of freedom, is an estimate of a variance with precision appropriate to a sample of size 2. Statistical methods appropriate to small samples are now well understood, but it still remains a fact that samples of size 2 from, say, a normal population with variance $\sigma^2$ will yield very varying estimates of $\sigma^2$, and with very poor precision. We may either say (1) that the square roots of the mean squares given in such a table as Brandt's Table 3 (there called variances) will, taking account of the sign of the effect and in the absence of real treatment effects, be normally distributed about zero with standard error best estimated, in this case, as the square root of the mean of the last nine items, and will therefore vary over a range of several times this standard error, or we may say (2) that the mean squares, on dividing by $\sigma^2$, are distributed as $\chi^2$ with 1 degree of freedom each. Tables of $\chi^2$, for this number of degrees of freedom, will equally show the wide range of variation that is possible for $\chi^2$ in a series of random samples of size 2 from a homogeneous normal population. The inference from this is that it may be dangerous to *select* those mean squares that appear to be significant at the 5% level, when examined by the $z$- or $F$-tests, and assert that these are real effects, without further examination of the question. The fact that on occasion significant *negative* interactions between two factors have been found by such methods, though usually passed over without explanation, should be a sufficient warning against attempting to explain the *positive* interactions that appear to exist, without confirmation in other ways. The writer has recently had an instructive example on this point, which will be referred to later on.

The modern method of calculating treatment effects, and the mean squares (1 D.F.) based on them, has rather directed attention away from the mean square for treatment generally, based upon all degrees of freedom available. With eight treatments, there will be a mean square based on 7 degrees of freedom, available for comparison by a $z$- or $F$-test with an error mean square, which, if there are four randomized blocks, will have 21 degrees of freedom. If the comparison shows that the treatment mean square is significantly larger than the error mean

square, then the hypothesis that treatments are without effect is disproved, and we are entitled to examine individual effects in relation to their standard error, a process which is exactly equivalent to testing the mean squares for the separate effects (1 D.F. each) against error by the $z$-test. For, when the $n_1$ of Fisher's (1936) table of $z$ is equal to 1, the $z$-test is equivalent to the $t$-test, with $n_2 = n$, and $z = \log_e t$. It has been urged on many occasions that the $z$-test for treatments as a whole should logically precede the $t$-comparison of treatment mean differences, for if the latter comes first there is a danger that certain differences may be asserted to be significant when they are in reality the differences of extreme members of a normally varying sample. Nevertheless, there was a reason for the decomposition of the total treatment mean square into component parts, for each comparison involved all plots, and it was quite likely that, in cases where the total mean square was insignificant, a significant effect might be missed through being watered down by inclusion with a number of insignificant effects. If considerably more than one-seventh of the treatment sum of squares is due to a single degree of freedom, the mean square for this effect may well be significant, even though a higher value of $z$ or $F$ is required, since $n_1$ is 1 and not 7.

In the type of calculation exhibited by Brandt, following Yates (1937), the above procedure is to some extent reversed, and even then it is hardly complete, for the separate effects are isolated and examined for significance, irrespective of whether or not the mean square for treatment generally is significant. With a considerable number of mean squares, based on a single degree of freedom each, it seems that the experimenter may sometimes be led astray. With, as in Brandt's case, fourteen effects to examine, we may easily have one or more reaching the 5 % significance level by pure chance, since this level denotes that the given $z$, or $F$, may be expected to reach this value by chance once in twenty times. For $n_1 = 1$, $n_2 = 9$ the 5 % $F$ is 5·12 and the 1 % value 10·56. Two out of fourteen effects are hailed as significant, with $F$-values 5·73 and 6·65 respectively, which exceed only by a little the 5 % significance level, and it is therefore possible that these may be chance results.

It is evident that some sort of supplementary test should be devised which will test whether the set of treatment mean squares, taken as a whole, are homogeneous or not. That the desirability of such a test is appreciated in certain circumstances is seen from Brandt's procedure when, in the absence of replication, he takes together the sum of squares for the 9 degrees of freedom of second and third order interactions to furnish a single mean square, which is taken as the estimate of error

variance. He states that it is proper to make a pooled estimate of variance only when the mean squares are homogeneous, and he proceeds to use a $\chi^2$ test for the purpose. The test he proposes is, however, not a very sensitive one. He gives the formula

$$\chi^2 = \frac{n^2 [S (s - \bar{s})^2]}{2 \{S (s)\}^2}$$

in which $s$ = mean square for an individual interaction, $\bar{s} = S (s)/n$, where $n$ = number of interactions involved, and he states that the table of $\chi^2$ is entered with $(n-1)$ degrees of freedom. This test is evidently based on the fact that, if a number $n$ of normally distributed variables $x$ have standard deviation $\sigma$, then

$$\chi^2 = \frac{S (x - \bar{x})^2}{\sigma^2},$$

where $\bar{x} = S (x)/n$, is distributed in the $\chi^2$-distribution with $(n-1)$ degrees of freedom. Brandt takes $x = s$, a mean square based upon 1 degree of freedom only, and therefore very unlikely to be normally distributed, since the usual assumption is that its square root is so distributed. He then uses the known result (Fisher, 1936)

$$\sigma_s^2 = \frac{2\sigma^4}{N-1}$$

for the variance of an estimate $s$ of variance based upon $N-1$ degrees of freedom, or derived from a sample of $N$ values of a normally distributed variable $x$, with variance $\sigma^2$. In the present case $N-1=1$, and the author further inserts for $\sigma^2$ the estimate $\{S (s)\}/n$ derived by pooling the $n$ interactions.

While Brandt correctly concluded that the mean squares were homogeneous, and could therefore be pooled, it is now suggested that a better test of the point at issue is one recently given by Bartlett (1937 *a*, *b*). The test is as follows:

To test the homogeneity of a set of $k$ estimated variances $s_r^2$, with $n_r$ degrees of freedom, obtain a "crude" value of $\chi^2$ by computing

$$n \log_e s^2 - \Sigma n_r \log_e s_r^2$$

or

$$2{\cdot}3026 \, (n \log_{10} s^2 - \Sigma n_r \log_{10} s_r^2),$$

where $s^2$ is the pooled estimate obtained from all taken together, with $n = \Sigma n_r$ degrees of freedom. If this value of $\chi^2$, which has $k-1$ degrees of freedom, is significant, it is advisable to calculate a "corrected" value by dividing by

$$C = 1 + \frac{1}{3(k-1)} \left( \Sigma \frac{1}{n_r} - \frac{1}{n} \right)$$

which much improves the $\chi^2$-approximation. Bartlett suggests that the application of the correction tends to over-correct the otherwise exaggerated significance of the "crude" $\chi^2$.

Applying this test to Brandt's error term, we have $n_r = 1$ for all $r$, and $k = 9$. $s^2$ is $37004/9 = 4111 \cdot 56$, and we find that the "crude" $\chi^2$ is 10·88, with 8 degrees of freedom. This value is not significant ($P > 0 \cdot 2$), so that there is no need to correct, but it is of interest to note that the "corrected" value of $\chi^2$ is 7·94. If this is an over-correction, then it is obvious that Brandt's value of 4·83 is much too low.

The new test is very simple to apply, and involves only a small amount of calculation on the logarithms of the mean squares, which should first be written down to base 10. (To work with natural logarithms is not so easy, owing to the wide range of figures customarily met with.)

The supplementary test that we have indicated above as necessary is evidently supplied by following Bartlett's procedure, and should be applied to the group of treatment mean squares. In fact, the whole group of twenty-three mean squares could be examined for homogeneity. Bartlett states that if possible heterogeneity between groups of variances is of special interest, the pooled variances for the different groups should be considered separately from the variation within groups, the "crude" values of $\chi^2$ being additive. The grouping should, however, be according to the lines prescribed by the experimental design. If, therefore, significant effects are suspected to exist within the group of main effects and first order interactions, the point may be tested by an examination of the first fourteen mean squares of the table for homogeneity. We find $s^2 = 7776 \cdot 89$ and $k = 14$, whence the "crude" $\chi^2$ is found to be 14·41 with 13 degrees of freedom. This value is far from being significant ($P > 0 \cdot 3$), and we may conclude that there is no reason whatever for picking out any of the single mean squares as abnormal. Thus no positive effects can with any degree of certainty be derived from the experiment at all, which is contrary to the author's conclusions, and the best that can be hoped for is that such effects as appear to exist may show up again in subsequent experiments, and hence gradually establish themselves as significant. The lack of conclusive results from this experiment is probably due to the exceedingly high error. If we base our error on the higher order interactions we have

$$s^2 = 4111 \cdot 56, \quad s = 64 \cdot 12 \text{ mg. of precipitate.}$$

Since the mean of the twenty-four flasks is $2442/24 = 101 \cdot 75$ mg. of

precipitate, we see that the standard error per flask is 63·0 %. The figures of Brandt's Table 2 range, indeed, from 0 to 291.

If Bartlett's test be applied to all twenty-three mean squares of Table 3 as one group, we find a "crude" $\chi^2$ of 26·33, which, with 22 degrees of freedom, gives a $P > 0{\cdot}2$. Thus the hypothesis that these are a set of twenty-three homogeneous values of $\chi^2$ is not disproved.

It is not claimed that such a test of homogeneity of estimated variances will be appropriate in all cases. It may sometimes be necessary to develop a test for the significance of the highest, say, of a number of mean squares. In this connection it may be said that when the error mean square is based on a large number of degrees of freedom, the highest among $k$ values of $F$, obtained by dividing each of the $k$ treatment mean squares by the error mean square, may approximately be tested with the help of the $\chi^2$-distribution: working to a 5 % level of significance the $P$-value found for $\chi^2 = $ maximum $F$ should be smaller than $1 - (0{\cdot}95)^{1/k}$ for the effect to be significant. The nature of this approximation is at present under examination.

*Plan of asparagus trial*

*Figures are numbers of sticks per plot*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| NPK 308 | K 295 | P 315 | N 241 | PK 303 | O 240 | NK 240 | NP 287 |
| NK 426 | NP 287 | PK 273 | O 289 | K 310 | P 314 | N 314 | NPK 266 |
| O 341 | NP 422 | NK 381 | PK 316 | NPK 353 | K 379 | P 378 | N 321 |
| N 403 | P 403 | K 371 | NPK 342 | NP 369 | O 323 | NK 366 | PK 351 |

As a further example we may take the case of an experiment on asparagus, now being carried out at the Kirton Agricultural Institute, and for the figures of which I am indebted to Mr J. C. Wallace. The experiment is of the simple N, P, K eight-plot type, with four replications of each of the eight treatment combinations. Confounding of the second order interaction was, however, introduced by keeping N, P, K and NPK in one group of treatments and O, NP, NK and PK in another group, thus permitting of the setting out of eight blocks. The diagram gives the manurial plan of the lay-out, together with the figures of numbers of sticks from a preliminary trial run, during which no treat-

ments were applied. As a uniformity trial, therefore, the analysis of variance scheme will be as follows:

| Variation | Degrees of freedom |
|---|---|
| Between blocks | 7 |
| Within blocks | 24 |
| Total | 31 |

It is instructive, however, to see what happens when the treatment effects are calculated as if the various treatments had been imposed. Counting the NPK interaction as confounded with blocks, we have six direct effects and first-order interactions, and the complete analysis of variance table is shown below.

| Variation | Degrees of freedom | Sum of squares | Mean square | |
|---|---|---|---|---|
| Blocks | 7 | 45,802·2 | 6543·2 | |
| N | 1 | 488·3 | 488·3 | |
| P | 1 | 69·0 | 69·0 | |
| K | 1 | 34·0 | 34·0 | |
| NP | 1 | 830·3 | 830·3 | |
| NK | 1 | 57·8 | 57·8 | |
| PK | 1 | 9,765·0 | 9765·0 | $z=0\cdot9743$ |
| [Total treatments | 6 | 11,244·4 | 1874·1] | |
| Error | 18 | 25,042·3 | 1391·2 | |
| Total | 31 | 82,088·9 | | |

The mean square for total "treatments" (6 D.F.) is not significant, but on examination of the separate effects it would appear that the PK interaction is significant at the 5 % level. The $z$ is 0·9742, compared with a 0·05 significance level of 0·7424 and a 0·01 level of 1·0572. With many ordinary experiments we should be happy to claim such a result as indicating a real effect, the nature of which is shown in the present case by the following table of totals of eight plots each:

| | Without K | With K | |
|---|---|---|---|
| Without P | 2472 | 2768 | Standard error 105·5 |
| With P | 2775 | 2512 | |

But since the treatments were not applied, the effect is entirely accidental. Using Bartlett's test on the six single degree of freedom mean squares, we find a "crude" value of $\chi^2$ of 11·30, with 5 degrees of freedom. The 5 % significance level is 11·07, so that $P$ is just under 0·05. The correction $C$ is $1+(6-\frac{1}{8})/15=1\cdot39$, and the "corrected" $\chi^2$ is 8·13, with a $P>0\cdot10$. It is clear, therefore, that the set of mean squares is compatible with the hypothesis that it is homogeneous, and awkward explanations are avoided.

It might be better to include the error mean square (18 D.F.) as a

seventh mean square in the homogeneity test. The result of doing so is to have a crude $\chi^2$ of 11·51, which is not significant ($P > 0{\cdot}05$). The other side of the picture is shown by an examination in the same way as the above of the yield figures (in lb.). It is not perhaps necessary to quote these in full, but it may be said that in this case there was a "significant" *negative* interaction, the mean square for NP being 0·01, while that for error was 5·57, resulting in a $z$ of $-3{\cdot}1613$, significant at the 5% level for $n_1 = 18$, $n_2 = 1$. This arose from the following totals of eight plots each:

| | Without N | With N | |
|---|---|---|---|
| Without P | 136·7 | 138·1 | Standard error 6·67 |
| With P | 131·7 | 133·5 | |

The homogeneity test on the six "treatment" mean squares gives a "crude" value of $\chi^2$ of 8·61, which is not significant ($P > 0{\cdot}10$).

The existence of these uniformity trial results will be taken account of when the actual results of the trial come to hand, and may serve to increase the precision of the experimental figures. But the point to be made at present is that "effects" which must be entirely chance results have been shown to pass the test of significance customarily employed in the analysis of field experiments of this type, which renders it necessary that some such test as that suggested for the homogeneity of a set of mean squares should invariably be employed to safeguard the conclusions of the experimenter.

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# EXCRETION OF NITROGEN BY LEGUMINOUS PLANTS[1]

By P. W. WILSON[2] and J. C. BURTON

## Introduction

Although mixed cropping has long been used in agriculture, little progress has been made toward an understanding of the origin of the benefits arising from the practice. For the special case of mixed cultivation of leguminous and non-leguminous plants, however, ample evidence exists to show that the non-legume may secure part of its supply of nitrogen from the legume after the latter, in association with the proper bacteria, has fixed free nitrogen from the atmosphere. For many years it was thought that the nitrogen received by the non-legume in the plant association originated from nodules and portions of the roots sloughed off the legume. Investigations on the problem by Lipman (1912) in the early part of this century led him to the conclusion that the legume may excrete nitrogenous compounds which can be utilized by the non-legume in the associated culture. Because other investigators failed to obtain comparable results in either field or greenhouse experiments, Lipman's work was practically disregarded for almost twenty years; then Virtanen and his collaborators in Finland (1933) reported similar findings. Since Lipman's experiments were made in open containers and extended over fairly long periods of time, the possibility remained that the observed benefit to non-legumes grown in association with legumes arose from non-symbiotic fixation by soil organisms; likewise, sloughing off of the root systems of the legume was not rigidly excluded as a source of the benefit. Virtanen and collaborators, however, in a series of ingenious and rigorous experiments (1935, 1937), eliminated these two alternative explanations and definitely established the fact that certain leguminous plants are able to excrete nitrogen from the nodules into the substrate (1935).

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[2] John Simon Guggenheim Memorial Foundation Fellow, 1936. Appreciation is expressed to the Foundation for their grant to Prof. P. W. Wilson to study fixation of nitrogen in consultation with European authorities. The experiments at Helsinki, Finland, were made while on this fellowship.

Thornton & Nicol (1934*a*, *b*) have reported positive results with respect to excretion by lucerne. No attempt will be made to review the literature concerned with the positive findings since several excellent reviews are available (Nicol, 1934, 1936; Virtanen, 1935). Instead, the negative results of other investigators will be presented, the implications of which, in the opinion of the authors, have not been sufficiently emphasized. Such emphasis is believed desirable at this time since, as a result of the numerous positive experiments of Virtanen and his collaborators, the idea is gaining credence that excretion of nitrogen by leguminous plants is a common occurrence in nature and *must* be the origin of benefit to non-legumes in mixed croppings.

Thus G. Bond (1936) in a paper concerned with transfer of nitrogen from nodules to plant in the soy-bean presents extensive calculations based on the assumption that the plants excreted part of the nitrogen fixed into the substrate. All reported work with this plant has been negative with respect to excretion; in a later paper Bond (1937) also reports negative findings when the assumption was tested in actual experiments. Loehwing (1937), discussing the experiments of Virtanen in a recent review, states: "It is evident from these data that any procedure endeavouring to employ only the nitrogen content of the nodules or even of the entire host plant as an index of efficiency, as has been done in the past, is wholly inadequate and misleading. Diffusion of amino nitrogen to the soil must also be determined if any comprehensive impression of the fixation processes is to be obtained." Although the desirability of testing for excretion in any physiological experiment is not denied, our own experience, as well as that of others, leads to the conclusion that excretion is the exception rather than the rule in experiments as they are ordinarily performed in the greenhouse, and that there is little reason to suspect past experimentation because of neglect of this factor.

Also, it is emphasized that for the primary question involved, viz. whether or not the benefit observed in the practice of growing legumes and non-legumes together in the field is due to nitrogen excreted by the latter, negative results are equally as important as are positive ones. For, if a large number of experiments, carried out at different stations in which recognized standard techniques are used, results in no evidence of excretion the importance, or even the occurrence, of the phenomenon in nature remains undetermined. Under these circumstances it would appear that special conditions are necessary for securing evidence of excretion. Until these conditions are so defined that duplication of the

results at will is possible in any experiment station, definite decision as to whether the phenomenon is more or less in the nature of a "greenhouse artifact" or has a real significance in agricultural practice must be reserved.

*Negative findings in the literature*

In the majority of Lipman's experiments (1912) a soil plus sand substrate was used, to which, in some cases, additional combined nitrogen was added. For controls the legume and non-legume were grown separately but seeded at twice the rate used in the mixed crop. In the calculation of the results it was assumed that the non-legume in the mixed culture took up exactly one-half as much combined nitrogen as when grown alone, and that any excess in the non-legume crop was to be ascribed to nitrogen excreted from the legume. Reflection shows that such an assumption may lead to error since in the associated culture it is highly probable that the oats secured more than their "share" of combined nitrogen, and that the peas made up the deficit by fixing additional nitrogen from the air. For example, in the 1908 experiments, 100 oats grown alone contained an average of 793 mg. nitrogen; in the mixed cultures with peas, 50 oat plants contained 617 mg. nitrogen. The argument runs that the proper share of the nitrogen in the soil of these 50 oats is 793/2 or 397 mg., and the difference between this figure and 617 represents nitrogen excreted by the peas. However, it is quite possible that the oats secured all of their 617 mg. from nitrogen originally present in the soil since this contained at least 793 mg. Lipman's own data show that the peas cannot successfully compete for combined nitrogen with the oats. When no $NaNO_3$ was added to the soil the peas comprised 44 % of the total crop (dry weight), but on addition of combined nitrogen the peas represented only 19 % of the total crop. For this reason experiments carried out in the presence of combined nitrogen cannot shed much illumination on the subject of excretion unless the non-legume takes up more nitrogen than was originally present in the combined form (Thornton & Nicol, 1934*a*). Field experiments suffer from this limitation and accordingly will not be considered in this paper.

In Lipman's experiments there were 26 cultures in which a nitrogen-free quartz sand substrate was used; in 20 of these no evidence of excretion was secured, while in 6 excretion was readily apparent. The following mixtures gave positive results: pea-oat, pea-barley, vetch-rye, vetch-barley and crimson clover-wheat. In other experiments, negative results were obtained with these same mixtures as well as with alsike

clover-millet, crimson clover-barley and soy-bean-corn (maize). Lipman could offer no definite explanation for these inconsistent results except to suggest that: "...Histological and physiological peculiarities in the plants themselves, as well as differences in the bacteria invading the legume roots, may be wholly or partly responsible for the results observed....The fact should be emphasized that in the study of reciprocal relations of legumes and non-legumes, it is not always easy to arrange the experiment so as to make the nitrogen supply the limiting factor of growth in each case."

Although Stallings (1926) concluded that $NH_3$ is the form of nitrogen transferred to the non-legume, examination of his data reveals no evidence of benefit to wheat when grown with the soy-bean. Recently, Ludwig & Allison (1937) described extensive experiments on the problem. The legumes used in the studies included cow-pea, soy-bean, lucerne, vetch, sweet pea, pea, sweet clover, red clover, bean and lespedeza; the non-legumes were Sudan grass, millet, pigweed, wheat, timothy, oats, barley, and Italian rye-grass. A large variety of environmental changes which would alter the rate and extent of growth were employed, but no evidences of excretion were obtained.

## Experimental

### *Wisconsin Experiment Station* (1933–6)

At the Wisconsin station the problem of associated growth of legumes and non-legumes has been under investigation since 1933. Wagner (1935) and V. S. Bond (1936) in numerous experiments studied a large number of possible factors which might influence the association, but in 15 experiments involving over 200 plant cultures, neither of these investigators was able to secure any evidence of excretion by the legumes used. Wilson (1935) discussed some of their data in a paper read before the American Society of Agronomy, but the results have never been completely published. Since all the experiments were negative in so far as excretion is concerned, only a summary of the factors varied in an effort to secure excretion will be presented.

(1) *Crop.* In the majority of the experiments a pea-oat mixture was used, hut in others a lucerne-rye-grass (Stallings, 1926; Thornton & Nicol, 1934*a*), as well as red clover and soy-bean without a companion crop, was included.

(2) *Variety of pea.* Alaska or Canada field pea was usually selected

for the trials, but other varieties including Strategem (U.S.), Nano Quarantino and Vittoria (Italy) and Torsdag (Sweden) were also used.

(3) *Strain of organism.* Two strains from the Wisconsin collection, *Rhizobium leguminosarum* 312 and 317, and one Finnish strain were tested.

(4) *Nutrient solutions.* Nitrogen-free Crone's solution (Hopkins *et al.* 1931) was used for the most part, but in some experiments Hiltner's solution (Virtanen & v. Hausen, 1935) or that used by Thornton & Nicol (1934*a*) was employed.

(5) *Sand.* Usually a local pit sand was used. This sand is not quite so fine as the quartz sand used by Virtanen and his associates, but it contains about 1 % clay which gives it high adsorptive capacity. It is practically free from nitrogen, contains limestone and possesses sufficient impurities that addition of the so-called "minor elements", such as molybdenum, boron and copper, is unnecessary. In all of our work at this station we have found it markedly superior to quartz sand as a substrate for nitrogen fixation studies. In some of the studies two different quartz sands, which were considerably coarser than the pit sand, were compared with the latter.

(6) *Aeration.* The effect of aeration on excretion was investigated by forcing air through the sand substrate by using containers of different volume or surface for a given number of plants, and by growing the plants in both glazed and unglazed pots.

(7) *Moisture.* In one experiment the moisture content of the pit sand and of one of the samples of quartz sand was varied from a fairly dry (10 %), through an optimum (15 %), to a saturated (20 %) level.

(8) $pCO_2$. In one experiment with red clover additional $CO_2$ was supplied to the plants.

(9) *Combined nitrogen.* Addition of small quantities of combined nitrogen in order to aid the initial development of the non-legume was practised in several experiments. Also, in some of the experiments just before the final harvest, when it was apparent that the non-legume was suffering from lack of nitrogen, combined nitrogen was added. In every case the development of the non-legume was definitely stimulated by the addition of combined nitrogen, indicating that it would have taken up nitrogen if the leguminous plant had been excreting it.

(10) *Time of seeding.* In a few experiments oats were seeded 1–2 weeks following the peas in order that nitrogen fixation by the legume would be under way before the nitrogen reserve in the non-legume was exhausted.

(11) *Time of harvest.* In all experiments in which the legume was

grown alone and detection of excretion depended on analyses of the sand, these were made at weekly intervals.

(12) *Ratio of legume to non-legume.* Ratios of one pea to from one to four oats have been used. Also, the total number of plants in a container of a given size has been varied.

The experiments in which these factors were varied, either singly or in combinations, were made during different seasons with consequent variation in extent and rate of fixation, but in no case was there observed any evidence of excretion.

*Biochemical Institute, Finland* (1936)

The results obtained at Wisconsin were discussed with Prof. Virtanen at the Second International Microbiological Congress at London in 1936. At his invitation one of us (P. W. W.)[1] visited his Institute in Helsinki and carried out experiments with a pea-barley mixture using both the "sterile" and non-sterile techniques (Virtanen, 1935; Virtanen *et al.* 1937). The details of the methods employed have been recently summarized by Virtanen *et al.* (1937). In the Wisconsin experiments only the non-sterile, i.e. open pot type of experiment had been used. The technique had differed from that employed in Prof. Virtanen's laboratory chiefly in the methods used to sterilize the seeds and sand. Dry sand was sterilized for 12 hr. at Wisconsin, while wet sand was sterilized for only 4 hr. at Helsinki; sodium hypochlorite was employed for sterilization of seed at Wisconsin (Hopkins *et al.* 1931); $HgCl_2$ was used at Helsinki (Virtanen *et al.* 1937).

The experiments were made in very cloudy weather which, together with the short day prevalent in Finland in the fall of the year, necessitated the use of artificial lights almost continuously. In spite of these handicaps definite evidences of excretion were obtained as can be seen by the data in Tables I and II. In these tables is given the actual quantity of nitrogen found in the *aliquot* of sand taken for analysis, since this figure is frequently of greater value in judging the significance of the result than is the calculated total for the entire lot of sand in the container. All the analyses of the sand which were made in duplicates by the method previously described (Virtanen & v. Hausen, 1935), checked

[1] I wish to express my appreciation to Prof. Virtanen for the many courtesies extended me while making the experiments at Helsinki, as well as for his kind co-operation with us before and since my stay there. Also my thanks are due to Dr Synnöve von Hausen and to Miss Saara Saastamoinen for their generous assistance in carrying out the experiments at Helsinki.

within 0·3 ml. $N/28$ acid. In some experiments the nutrient solution was removed from the sand by suction and analysed separately; in others, the sand was dried before analysis.

Table I. *Excretion of nitrogen by peas alone and in association with barley (Helsinki experiment, sterile container)*

| Treatment | Nitrogen in peas mg. | Nitrogen in barley mg. | Nitrogen in substrate: Liquid mg. | Nitrogen in substrate: Aliquot of sand* mg. | Nitrogen excreted: Total | Nitrogen excreted: %† | Remarks |
|---|---|---|---|---|---|---|---|
| Exp. I: | | | | | | | |
| Harvest 1 | | | | | | | |
| Control | 12·9 | — | 0·30 | 0·63 | — | — | |
| *HX* | 19·3 | — | 4·00 | 2·15 | 14·2 | 69 | 2 Torsdag peas alone |
| PP | 31·2 | — | 3·12 | 1·61 | 10·7 | 37 | |
| Harvest 2 | | | | | | | |
| Control | 11·5 | — | 0·50 | 0·60 | — | — | |
| *HX* | 36·9 | — | 6·05 | 2·57 | 19·9 | 44 | 2 Torsdag peas alone |
| PP | 53·1 | — | 1·20 | 1·50 | 7·25 | 25 | |
| Control | 8·7 | 1·30 | 0·10 | 0·60 | — | — | |
| | 5·8 | 1·15 | 0·10 | 0·50 | — | — | |
| *HX* | 21·2 | 4·5 | 1·85 | 0·80 | 7·10 | 33 | 1 Torsdag pea and |
| | 26·9 | 3·7 | 0·50 | 0·70 | 4·80 | 20 | 1 Binder barley |
| PP | 29·0 | 2·4 | 0·35 | 0·75 | 2·95 | 12 | |
| | 37·7 | 2·6 | 0·30 | 0·70 | 2·70 | 8 | |
| Exp. II: | | | | | | | |
| Control | 11·65 | 3·22 | — | 0·60 | — | — | 2 Torsdag peas and |
| *HX* | 47·2 | 4·06 | — | 0·85 | 4·5 | 13 | 2 Binder barleys |
| | 110·7 | 8·12 | — | 0·65 | 5·6 | 6 | |
| *HX* | 91·6 | 5·32 | — | 0·95 | 8·9 | 10 | 1 Torsdag pea and |
| | 66·6 | 4·20 | — | 0·60 | 2·6 | 4 | 1 Binder barley |

Exp. I. Sown, 20 Oct.; Harvest 1, 12 Nov.; Harvest 2, 22 Nov. 1936. 2300 g. sand in suction flask.

Exp. II. 21 Oct. to 14 Dec. 1936. 4500 g. sand in Woulff bottles.

* 200 g. in Exp. I; 300 g. in Exp. II.

† Percentage of total nitrogen fixed is obtained from: (N excreted) divided by (N fixed in plant + N excreted).

In Exp. I the evidence for excretion is clear-cut, especially with the Finnish culture H*X*; culture PP had previously been found to excrete less than did H*X* (Virtanen *et al.* 1937). Although the total quantity of nitrogen fixed by the peas was rather small, the *relative* amount excreted compares quite favourably with that previously reported by Virtanen and associates (1937). The barley plants grown with the inoculated pea plants were dark green and considerably taller than those grown with the uninoculated controls. In Exp. II, which wa maintained for a longer period of time than was Exp. I, the *total* quantity of nitrogen fixed by the legume was higher, but the *relative* quantity of nitrogen

excreted was considerably less. Exp. III, made in open containers, had to be harvested about a week after nitrogen fixation had started as judged by the appearance of the plants. At this time the barley and oats grown in association with the peas were distinctly superior to those grown alone. Although analysis confirmed in every case the greater nitrogen content of the non-legume grown in the mixture over that of the non-legume grown alone, the quantity of nitrogen involved is too small to allow definite conclusions. Other experiments were made at Helsinki using in addition to the Finnish bacteria and varieties of plants, Canada field pea and strains of *Rhizobium leguminosarum* from the Wisconsin collection (Nos. 312 and 317). The growth of the plants was slow, and the results were erratic; in some cases excretion was evident, in others it was absent.

Table II. *Excretion of nitrogen by peas in presence of non-legume (Helsinki experiment, open containers)*

| Treatment | Nitrogen in 5 peas mg. | Nitrogen in non-legumes mg. | Nitrogen in sand Aliquot* mg. | Nitrogen excreted Total mg. | Nitrogen excreted % | Remarks |
|---|---|---|---|---|---|---|
| Exp. III: | | | | | | |
| Control (barley) | — | 19·5 (9·8)‡ | 0·85 | — | — | 12 Binder barleys alone |
| Control (peas)† | 83·9 | 10·9 | 1·10 | 9·5 | 15 | 6 Binder barleys plus 5 Torsdag peas |
| HX | 112·1 | 11·1 | 0·95 | 5·2 | 6 | 6 Binder barleys plus 5 Torsdag peas |
| PP | 53·5 | 14·7 | 0·85 | 9·8 | 29 | 5 Binder barleys plus 5 Torsdag peas |
| 317 | 51·0 | 11·4 | 0·75 | 2·2 | 9 | 6 Binder barleys plus 5 Torsdag peas |
| Control (oats) | — | 17·1 (8·6)‡ | 0·75 | — | — | 12 Guldregn oats alone |
| Control (peas) | 65·2 | 9·1 | 1·05 | 10·0 | 22 | 6 Guldregn oats plus 5 Torsdag peas |
| HX | 72·8 | 10·4 | 0·90 | 9·2 | 18 | |
| 312 | 52·9 | 10·0 | 0·90 | 8·7 | 27 | |

* 300 g.

† Became contaminated; fixation estimated on basis of 6·0 mg. N per pea (average in other experiments).

‡ Figures in parentheses are for 6 plants, number used in mixed cultures.

Exp. III. 19 Oct. to 20 Nov. 1936. 9 kilos sand in porous pots.

### *Wisconsin Experiment Station* (1937)

The experiments made at Helsinki were repeated and extended the following spring at the Wisconsin experiment station in Madison. The technique of Virtanen and associates was carefully followed in every detail so that experiments were available in which the variety of barley

and pea, the strain of organism, the type of container, and the nutrient solution were identical with those used in the Helsinki experiments. The Finnish methods for sterilization of seed and sand likewise were employed. In addition, plant cultures were included which duplicated those made at this station in 1933–6 with respect to use of Wisconsin varieties of bacteria and seeds. In all experiments, however, the method for sterilizing sand used at Helsinki was followed, since preliminary trials indicated that the growth of the plants was better if a shorter period of sterilization was used.

The aseptic transfer of the seedlings of barley and pea for the "sterile" technique (Virtanen *et al.* 1937) was made in the transfer chamber previously described (Hopkins *et al.* 1931). In general, these experiments included some which were exact duplicates of those made in Helsinki except that American types of sand were used and the general environment, e.g. greenhouse and weather, were necessarily different.

Virtanen *et al.* (1937) have recently published accounts concerning the importance of the adsorptive properties of the substrate employed for securing excretion. It is of interest to compare the physical properties of the sands used with those of the Finnish sand. These are summarized in the following table:

*Physical properties of sands*

I. Mechanical analysis.

| Mesh | Name | Size | Finnish Quartz % | American Quartz % | American Pit % |
|---|---|---|---|---|---|
| 10 | Fine gravel | 2 –1 mm. | 0·0 | 0·0 | 2·5 |
| 20 | Coarse sand | 1 –0·5 mm. | 0·1 | 25·7 | 16·5 |
| 40 | Medium sand | 0·5 –0·25 mm. | 8·9 | 56·5 | 37·5 |
| 60 | Fine sand | 0·25–0·1 mm. | 55·9 | 11·8 | 22·1 |
| 80 | Very fine sand | 0·1 –0·05 | 29·7 | 4·1 | 11·9 |
| 100 | Silt and colloid | 0·05– | 4·6 | 2·4 | 8·5 |
| | | | 99·2 | 100·5 | 99·0 |

II. Water relations:

| | Finnish Quartz % | American Quartz % | American Pit % |
|---|---|---|---|
| Moisture holding capacity | 24·0 | 22·7 | 23·3 |
| Moisture equivalent | 4·3 | 3·3 | 6·3 |

The data which deal with particle size indicate that the Finnish quartz is definitely finer than the American quartz used and somewhat finer than the pit sand. However, the adsorptive capacity of the latter, (moisture equivalent) as measured by the quantity of moisture retained after 15 min. suction on a water pump (Bouyoucos, 1935), is considerably higher than that of the Finnish sand, probably because of the higher content of colloidal material in the pit sand.

The greenhouse at Helsinki is located on a roof six stories above street level, and provision is made to move the cultures into the open air during favourable weather. The location and arrangement provide excellent facilities for lighting and temperature control. The greenhouse at Madison is of the usual type but it has been provided with special devices for control of environment (Wilson & Georgi, 1932). In view of the recent statement of Virtanen *et al.* (1937) that high humidity favours excretion, it should be mentioned that provision is made in the Madison greenhouse to keep the floor and benches sprinkled with water so that

Table III. *Excretion of nitrogen by peas alone and in association with barley (Madison experiments, sterile container)*

| Treatment | Nitrogen in peas mg. | Nitrogen in barley mg. | Nitrogen in substrate: Liquid mg. | Nitrogen in substrate: Aliquot of sand* mg. | Nitrogen excreted: Total | Nitrogen excreted: % | Remarks |
|---|---|---|---|---|---|---|---|
| Exp. IV: | | | | | | | |
| Control | 9·9 | — | 0·76 | 1·0 | — | — | 2 Torsdag peas in Quartz sand No. I |
| | 10·0 | — | 1·46 | 0·6 | — | — | |
| HX | 76·0 | — | 2·64 | 0·7 | 0·3 | 0 | |
| | 81·0 | — | 2·39 | 1·6 | 9·0 | 10 | |
| Control | 4·55 | 3·54 | 1·83 | 0·3 | — | — | 1 Torsdag pea and 1 Binder barley in Quartz sand No. I |
| | 7·31 | 1·65 | 1·76 | 0·8 | — | — | |
| | 4·80 | 2·10 | 0·85 | 0·6 | — | — | |
| HX | 36·1 | 4·5 | 2·10 | 3·0 | 29·0 | 49 | |
| | 29·6 | 5·1 | 2·14 | 3·4 | 35·7 | 64 | |
| | 32·7 | 4·5 | 2·43 | 1·4 | 12·0 | 31 | |
| Exp. V: | | | | | | | |
| Control | 8·9 | — | 1·06 | 1·6 | — | — | 2 Canada field peas alone in Quartz sand No. I |
| 317 | 88·0 | — | 2·25 | 2·4 | 10·7 | 12 | |
| | 67·0 | — | 2·05 | 1·4 | 0·0 | 0 | |
| Control | 6·7 | 2·7 | 0·2 | 1·3 | — | — | 1 Canada field pea and 1 *Wis.* 38 barley in Quartz sand No. I |
| 317 | 36·5 | 7·1 | 3·4 | 2·8 | 26·0 | 47 | |
| | 54·3 | 8·6 | 2·5 | 3·0 | 27·4 | 37 | |
| Exp. VI: | | | | | | | |
| Control | 8·9 | — | — | 0·8 | — | — | 2 Torsdag peas in Quartz sand No. 2 |
| HX | 37·8 | — | — | 1·9 | 10·5 | 27 | |
| | 34·3 | — | — | 1·4 | 4·8 | 16 | |
| Control | 11·1 | — | — | 1·2 | — | — | 2 Canada field peas in Quartz sand No. 2 |
| 317 | 41·3 | — | — | 1·6 | 3·9 | 11 | |
| Control | 7·9 | — | — | 1·2 | — | — | 2 Torsdag peas in Pit sand |
| HX | 35·6 | — | — | 2·0 | 8·7 | 24 | |
| | 29·1 | — | — | 2·8 | 17·6 | 46 | |
| Control | 10·9 | — | — | 1·3 | — | — | 2 Canada field peas in Pit sand |
| 317 | 38·4 | — | — | 2·4 | 11·8 | 30 | |

* 300 g.
Exp. IV. 3 Mar to 29 Apr. 1937. 3000–3300 g. sand in suction flask.
Exp. V. 10 Mar. to 25 May 1937. 3000–3300 g. sand in suction flask.
Exp. VI. 20 May to 10 June 1937. 3000–3300 g. sand in suction flask.

Table IV. *Effect of type of sand, culture of bacteria and species of plants on association (Madison experiments, open container)*

| Culture | Type of sand | Nitrogen fixed mg. | Nitrogen in barley mg. | Increase from Association: Total mg. | Increase from Association: %* | Remarks |
|---|---|---|---|---|---|---|
| Exp. VII: | | | | | | |
| Control | Q | — | 4·15 | — | — | 4 Binder barleys—Hiltner's nutrient solution |
| | | | 3·98 | — | — | |
| | P | — | 3·11 | — | — | |
| | | | 3·40 | — | — | |
| HX | Q | 392·4 | 3·16 | − 0·90 | — | 3 Torsdag peas and 4 Binder barleys—Hiltner's nutrient solution |
| | | 305·7 | 2·69 | − 1·37 | — | |
| | P | 547·6 | 3·74 | + 0·49 | — | |
| | | 532·6 | 5·30 | + 2·05 | — | |
| 317 | Q | 446·4 | 4·19 | + 0·13 | — | |
| | P | 441·2 | 4·67 | + 1·42 | — | |
| | | 395·7 | 3·99 | + 0·74 | — | |
| Control | Q | — | 4·48 | — | — | 4 *Wis.* 38 barleys—Crone's nutrient solution |
| | | | 5·20 | — | — | |
| | P | — | 3·52 | — | — | |
| | | | 3·77 | — | — | |
| HX | Q | 70·9 | 5·40 | + 0·56 | — | 4 Canada field peas and 4 *Wis.* 38 barleys—Crone's nutrient solution |
| | | 121·5 | 6·86 | + 2·02 | — | |
| | P | 146·8 | 4·30 | + 0·65 | — | |
| | | 268·3 | 4·53 | + 0·88 | — | |
| 317 | Q | 382·2 | 4·20 | − 0·64 | — | |
| | | 141·6 | 3·60 | − 1·24 | — | |
| | P | 449·0 | 4·89 | + 1·25 | — | |
| | | 451·0 | 3·96 | + 0·71 | — | |
| Exp. VIII: | | | | | | |
| Control | Q | — | 4·9 | — | — | 5 Binder barleys—Hiltner's nutrient solution |
| | | | 6·2 | — | — | |
| | P | — | 7·7 | — | — | |
| | | | 8·2 | — | — | |
| HX | Q | 186·0 | 12·9 | + 7·4 | 3·8 | 4 Torsdag peas and 5 Binder barleys |
| | | 278·7 | 10·5 | + 5·0 | 1·3 | |
| | P | 419·2 | 26·6 | +18·7 | 4·3 | |
| | | 297·6 | 25·9 | +18·0 | 5·6 | |
| 317 | Q | 283·0 | 6·7 | + 1·2 | — | |
| | | 214·6 | 6·0 | + 0·5 | — | |
| | P | 320·0 | 8·6 | + 0·7 | — | |
| | | 378·4 | 8·0 | + 0·1 | — | |
| Control | Q | — | 8·6 | — | — | 5 *Wis.* 38 barleys—Crone's nutrient solution; 4 Canada field peas and 5 *Wis.* 38 barleys—Crone's nutrient solution |
| | | | 5·9 | — | — | |
| | P | — | 9·2 | — | — | |
| | | | 8·8 | — | — | |
| HX | Q | 220·5 | 9·2 | + 1·9 | — | |
| | | 141·7 | 9·5 | + 2·2 | — | |
| | P | 357·6 | 9·9 | + 0·9 | — | |
| | | 258·0 | 10·9 | + 1·9 | — | |
| 317 | Q | 96·0 | 28·3 | 21·1 | 18·2 | |
| | | 183·2 | 10·6 | 3·4 | — | |
| | P | 227·2 | 8·1 | − 0·9 | — | |
| | | 339·4 | 16·9 | + 7·9 | 2·2 | |

Q = Quartz sand No. 2; P = Pit sand.
Exp. VII. 15 Feb. to 28 Apr. 1937. 3300 g. sand in small unglazed pots.
Exp. VIII. 28 Feb. to 19 May 1937. 9000 g. sand in large unglazed pots.
* Percentage of total nitrogen fixed is obtained from: (N increase in barley) divided by (N fixed by pea + N increase in barley).

the relative humidity is maintained at approximately 70–80%. In contrast to the unfavourable weather experienced during the fall experiments at Helsinki, the spring weather at Madison was ideal for the development of plants. The peas fixed large quantities of nitrogen, and their general development was quite comparable to those grown in the field. Excellent growth was obtained especially in the open pots; in these the peas reached a height of 6 to 7 ft., and bore well-filled pods at harvest.

In spite of the excellent development of the plants excretion was not always observed, as can be seen from the data in Tables III and IV. In the experiments in which "sterile" plant cultures were used, definite evidences of excretion were obtained in 10 or 15 cultures examined. In two cultures the results were definitely negative, and in three others, doubtful. As far as these data are concerned, the phenomenon was not especially influenced by type of sand, strain of bacteria, or variety of pea or barley used. The presence of barley, however, did appear to stimulate the excretion; the only clear-cut excretion in the absence of barley was in Exp. VI, which was an extremely short-time one (Virtanen & v. Hausen, 1935).

In the open pot experiments there are even greater inconsistencies in the occurrence of excretion. Even though the growth of the peas in Exp. VII was excellent, and the quantity of nitrogen fixed was two or three times that observed in the "sterile" containers, excretion of nitrogen was not obtained in any case. Although the relative quantity of the total nitrogen fixed which was transferred to the barley was much less than has been noted by Virtanen and his collaborators, the general appearance of the plants, as well as the nitrogen determinations, left no doubt that the barley plants in the positive cultures benefited through the association. Of interest was the lack of agreement between duplicate cultures in two cases; a definite benefit to the barley was observed in one culture but not in its duplicate. As can be seen from the data, the agreement with respect to nitrogen fixed was better in the cultures grown in the pit sand; in the quartz sand the development of the peas lagged in the initial stages, and erratic results in the final quantity of nitrogen fixed were frequently obtained.

## Discussion

From a consideration of the experience of the several investigators on the problem of excretion of nitrogen by leguminous plants, it is apparent that two facts have been established: (*a*) under certain conditions

excretion of nitrogen from the nodules of the leguminous plant occurs, and this excretion may account for a considerable portion of the total nitrogen fixed; (b) excretion does not always accompany fixation, and, judging from the majority of reports in the literature, it appears to be the exception rather than the rule in greenhouse studies.

It is obvious that the phenomenon of excretion depends on the realization of certain conditions which affect the equilibrium between the nitrogen in the substrate and in the nodules. It is equally obvious that the necessary conditions have not been completely defined, otherwise duplication of the results would be an easy matter in any experiment station since the general technique is quite simple. Strain of organism, variety of plants, type of container and nature of substrate[1] undoubtedly affect the quantitative relationships, but they do not appear to determine entirely whether or not excretion will occur. In the experiments reported in this paper, both positive and negative results have been obtained when these factors were unchanged.

The chief reason that has occurred to the writers for the discrepancies among similarly treated cultures in the same or in different experiments is the inability to control the general development of the plants, especially in their relation to one another. It has been our observation that, whenever excretion has occurred, the legume and non-legume have developed uniformly. For example, if weather conditions are unfavourable, fixation of nitrogen by the leguminous plant is poor, and no excretion takes place. On the other hand, if the weather is excellent, the legume may develop too rapidly, and again there is no excretion; apparently, under these conditions the legume utilizes the nitrogen as it is fixed, and none accumulates to be excreted. In the experiments conducted at Madison in the spring an effort was made to slow down the development of the plants in Exp. VIII by maintaining a fairly low temperature in the greenhouse. It will be noted that excretion occurred in this experiment but did not in Exp. VII, in which the growth of the peas, especially in the early stages of their development, was unchecked. This effect of temperature is under further investigation. Perhaps it is no coincidence that the most consistent results have been obtained with

[1] It is doubtful whether the small differences among the sands which were used in the experiments described could account for the absence of excretion in most of the work at the Wisconsin Experiment Station. If this were so, then the general occurrence of the phenomenon in nature would be open to serious question as it would appear to depend on the presence of a rather specific type of substrate. In order to settle this point definitely, Prof. Virtanen has arranged to supply us with some Finnish sand which will be compared with the American sands in future experiments.

the so-called cool-weather plants (Ludwig & Allison, 1937) rather than with the cow-pea and soy-bean plants, which grow and mature quite rapidly.

The occurrence of excretion can usually be detected in the appearance of the pea plants as well as in that of the barley or oat plants in association. If excretion occurs, as judged by the development of the non-legumes, the growth of the peas lags behind that of peas in companion cultures which are not excreting. Later, the peas which excrete may overcome the initial difference, but this restriction of the early growth of the legume has been consistently observed. Finally, the intimate interdevelopment of the roots should be mentioned (Nicol, 1934, 1936). In our own experiments, whenever excretion bas occurred, separation of the roots of the legume and non-legume was most difficult, although this was comparatively easy in the absence of excretion. Whether this is a cause or an effect of the transfer of nitrogen, cannot yet be determined.

In conclusion, it is apparent that our knowledge of excretion of nitrogen by leguminous plants has yet to reach the stage at which definite statements regarding its significance in the field can be safely advanced. Virtanen and his collaborators have had no difficulty in repeating their experiments, but there still remains a considerable body of negative data which is unexplained. It is suggested that a real service will be rendered practical agriculture if speculations regarding the significance of present findings be reserved until additional knowledge makes it possible to determine at least the probability that the phenomenon actually occurs under natural conditions in the field.

## Summary

The literature which deals with the question of excretion of nitrogen by leguminous plants is reviewed especially with reference to accounts of experiments in which no excretion was obtained.[1]

Experiments made at the Wisconsin experiment station prior to 1936, primarily concerned with the associated growth of pea and oat mixtures, were uniformly negative with respect to benefit to the oat plants through the association.

Experiments carried out at the Biochemical Institute in Helsinki, Finland, with a pea and barley mixture were in general positive, although poor development of the plants because of unfavourable weather were accompanied by little or no excretion.

[1] Romashev's (1936) entirely negative results are not, however, discussed here.

On duplicating the Helsinki experiments at Wisconsin, once more both positive and negative results were obtained, although the development of the plants was excellent.

The occurrence of excretion appears to depend on many complicated circumstances some of which have been defined, while others are at present unknown. Factors which insure a uniform development of the plant cultures and especially a not too rapid growth of the leguminous plant appear to be important; as yet no universally successful method of achieving this type of development has been determined. It is concluded that although there can be no doubt with regard to the existence of excretion, knowledge of the factors concerned is still insufficient to allow duplication of the results consistently at all experiment stations. Until such knowledge becomes available, application to practical agriculture can remain only an attractive possibility and not an established fact.

### NOTE ADDED TO MANUSCRIPT

Since submitting this manuscript a few other papers of unusual interest have appeared:

(1) Bond, G. (*Nature*, **140**, 683, 1937) in a preliminary report states that he has obtained negative results with respect to excretion with soy-bean, broad bean and only slight excretion with pea.

(2) Nowotnówna (*J. agric. Sci.* **27**, 506, 1937) reports experiments performed in Poland, in which rye grass derived benefit through association with peas, clover and serradella. Peas were superior to the other two legumes as a source of benefit. On the other hand, when barley was used as the indicator plant in experiments at Rothamsted, benefit was observed with peas, not with lucerne or clover. All these experiments were in open pots and long-time in nature, so that effects other than excretion may have played a role. She interprets her data to indicate that plants of similar growth habit must be employed in mixed cropping experiments, a conclusion which appears consistent with that reached in this paper; namely, the necessity of a uniform development of the plants for the securing of excretion.

The recent comment of Virtanen (*Nature*, **140**, 683, 1937) on our negative experiments apparently rests upon a misunderstanding of our experimental conditions. He states: "When Prof. Wilson could find no excretion in his numerous experiments...his experimental conditions must in some way differ from the natural ones. So far as is known to me, Prof. Wilson is using a very coarse quartz sand as the substrate and the

pot cultures are watered many times a day." As is indicated in this paper, the majority of our experiments have been made with "pit sand" and not with a quartz sand. This pit sand was specifically selected because it has been found after many years of trial at this station to be quite superior to other sands for allowing a more "natural" growth of plants; i.e. most nearly approximating growth in soil. It certainly is a substrate which, both physically and chemically, more nearly resembles soil than does pure quartz sand. Watering of the plants is practised here exactly as at Helsinki, viz. whenever the plants require it and not necessarily many times a day. Moreover, as is indicated in this paper, we investigated in our early experiments the influence of moisture content and showed that this factor was not the cause of our negative results. Virtanen's objections break down in any case, since we have secured both *positive* and *negative* results under the experimental conditions to which he directs his attention.

A second statement made in that note cannot be allowed to pass unchallenged. It is stated: "On the basis of these experiments, and particularly of those carried out in the soil, *it can be definitely concluded that the excretion is a phenomenon which must occur in the field and plays an important part in practical agriculture.*" (Virtanen's italics.) Apart from the fact that the numerous negative results by other investigators show that the phenomenon does not invariably occur even in the greenhouse, it should be recognized that the open pot experiments and certainly the sterile culture ones, are artificial as far as field conditions are concerned. Unfortunately, experiments in the field cannot unequivocally prove that excretion has occurred, since other factors, e.g. stimulation of free-living nitrogen fixers, sloughing-off of plant tissue, and increasing availability of the soil nitrogen to the non-legume by reason of the growth of the legume, may exert an influence similar to excretion. The same is true of greenhouse experiments in which soil is used as a substrate. The most that can be said is that, as a result of the greenhouse experiments, there is reason to believe that excretion may play a role under certain conditions in the well-recognized benefits of field cropping of mixtures of legumes with non-legumes. Other factors, undoubtedly, are important, as is evidenced by beneficial effects obtained when two non-legumes are used in mixed cropping. To state dogmatically that excretion must occur under field conditions is, in our opinion, an unwarranted conclusion from present experimental data.

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# THE PROXIMATE ANALYSIS OF THE ORGANIC CONSTITUENTS IN NORTH-EAST SCOTTISH SOILS, WITH SOME NOTES ON THE METHODS

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(With One Text-figure)

MUCH analytical work has been undertaken in the profile examination of soils, but so far as Scottish soils are concerned no proximate analyses of the organic constituents have been recorded. The present investigation was carried out on six typical forest and heath soil profiles, and records determinations of the various organic constituents throughout the different soil horizons. The method of proximate analysis worked out by Waksman & Stevens (1930*a*, *b*) was adopted as a basis for the experimental method. Preliminary experiments conducted here as well as elsewhere (Tiurin & Kononova, 1934) showed that the method required careful examination before it could be applied unconditionally to all soils. In brief, the following results were obtained, and the method of Waksman & Stevens (1930*b*) modified accordingly.

### (1) *Ether-alcohol-water extracts*

In the ether extractions much longer periods were necessary before concordant results were obtained, and a period of at least 24 hr. was found essential. Further, the extraction of the mineral layers of the profile with boiling water was practically impossible owing to filtration difficulties.

### (2) *Hemicellulose*

Of the many methods devised (Tollens & Krober, 1902; Dickson *et al.* 1930; Waksman & Reuszer, 1932), it is recognized that no single one can give a complete estimation of the total hemicellulose present. The hydrolysis method of Waksman has been used here, it being expressly understood that this determines those hemicelluloses (cellulosans and polyuronides) whose sugar products on hydrolysis are not destroyed by the acid treatment. How far such a measurement is a true estimate of the hemicellulose present is impossible to say.

*Estimation of reducing sugars*

In view of the strong criticism of Amick (1927), as well as the experience gained in the course of this work, it was decided to use the much more satisfactory and quicker method of Lane & Eynon (1923). In applying either Lane & Eynon's or Bertrand's (1906) method (as used by Waksman) to soil solutions, additional difficulties were experienced. These were due to the effect of humus, various dissolved salts, etc. on the sugar estimations, some causing positive errors (e.g. manganese, etc.), some negative (dilution, calcium, etc.). The method finally adopted for the determination of reducing sugars was as follows:

10–25 c.c. of the acid hydrolysate were neutralized, made alkaline to brom-cresol-purple with 2·5 % NaOH, allowed to stand for a few hours with frequent shaking, after which the iron-manganese precipitate was filtered and washed and the filtrate made up to 100 c.c. (In some cases two precipitations were necessary.) Fehling's solution was added (Lane & Eynon's A and B), the mixture boiled and a standard glucose solution was run in until an end-point was reached, using 3 drops of 1 % methylene blue as indicator. The whole operation must be completed 3 min. after boiling commences. The standard solution was treated in the same manner.

(3) *Cellulose*

In the use of the method of Kiesel & Semiganovsky (1927) for the estimation of cellulose, Waksman does not control the temperature. Since this is of importance, especially if lignin is to be estimated on the residues, and since no data could be found on this subject, it was decided to test it out on some cellulosic soil material. For this purpose partially decomposed pine needles were used after the removal of the portion soluble in ether-alcohol-water and the hemicellulose. Two strengths of acid were used, 72 and 80 %, in the ratio of 25 c.c. acid to 2 g. portions of the original material through a temperature range of 8–55° C. for 2½ hr. The reducing value was determined in the hydrolysate after dilution to 4 % and boiling 5 hr.—the results are set out graphically. The results of Ritter *et al.* (1933), which appeared while this work was in progress, are given for comparison. It will be seen that the effect of temperature is most marked, and since room temperatures under a period of observation were found to vary within the limits of 10–27° C. (in U.S.A. 18–40° C. were recorded), the importance of working at optimum conditions need hardly be stressed.[1]

[1] Since the completion of this work a paper has appeared in which the optimum conditions recommended are 72 % $H_2SO_4$ for 5–6 hr. at 10° C. (Bamford & Campbell, 1936).

In order to have some check on this hydrolytic method of cellulose estimation it was decided to determine cellulose in the organic layers of the soil profile by the usual Cross and Bevan chlorination method as previously used on these materials by Grosskopf (1926). The particular method used was that of Sieber & Walter (1913) with some modifications

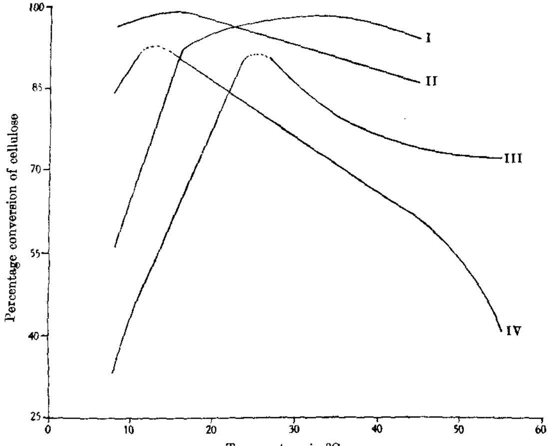


*Fig. 1. Effect of temperature and concentration of acid on conversion of cellulose to glucose.*

I. 72% $H_2SO_4$, 2 hr. standing } Ritter, Mitchell & Seborg.
II. 72% $H_2SO_4$, 6 hr. standing }
III. 72% $H_2SO_4$, 2½ hr. standing.
IV. 80% $H_2SO_4$, 2½ hr. standing.

recommended by Johnsen & Hovey (1918). The results are given in Table I along with those obtained by hydrolysis. It will be noted that in every case the percentage cellulose is higher by the chlorination method than by hydrolysis, although it must be remembered that in the chlorination method the acetic acid used for the removal of hemicellulose may be less drastic in its action than 2% HCl. An interesting feature was noted in the cellulosic residues from the organic layers of the

profiles. Whilst the cellulose in the loose leaves was white in appearance, that in the "F" layer was greyish, and that in the "H" was black, colloidal, and so difficult to filter that accurate determination was made impossible. Grosskopf, who found a similar phenomenon in the pine profiles investigated by him, did not consider the black residue to be true humus but humoid in nature (cf. Thaysen *et al.* 1926).

Table I. *Cellulose content of various organic soil horizons (expressed as percentage of the oven-dry materials)*

| Soil horizon | | Cellulose % | |
|---|---|---|---|
| | | By chlorination | By hydrolysis |
| Dinnet Moor | "LL" | 13·93 | 8·99 |
| | "F" | 7·65 | 6·30 |
| Potarch birch | "LL" | 29·18 | 12·03 |
| | "F" | 7·00 | 6·58 |
| Fintray beech | "LL" | 17·70 | 13·26 |
| | "F" | 9·26 | 8·21 |
| Aboyne oak | "LL" | 27·42 | 20·11 |
| | "F" | 14·72 | 14·40 |

In the mineral soil where the chlorination method is always inaccurate and often impracticable, the estimation of cellulose is best performed by hydrolysis, when it must be remembered that the values are probably always slightly low.

### (4) *Lignin-protein complex*

The separation of cellulose and lignin by the use of 80% sulphuric acid strongly affects the lignin and probably the humus. This was clearly shown when attempts were made to chlorinate the residue after cellulose hydrolysis on leaves and wood meal. Previous to treatment with concentrated acid (80% $H_2SO_4$), chlorination proceeded rapidly and effectively, leaving the pure cellulose. On separating the cellulose first, however (by hydrolyses with 80% acid), and then chlorinating, the process was exceedingly slow and there was left a black residue, amounting in some cases (e.g. oak leaves) to over 6% of the original material treated.

The conclusion seems obvious that the conditions required to give complete hydrolysis of cellulose are not those causing the least change in lignin residues, and whilst this may be of little importance in most soils, it is certainly an important factor in those which are mainly organic in nature.

The procedure finally adopted for the analysis of the organic layers of the soil profile was as follows:

*Carbon, nitrogen, moisture and acidity determination.* On separate portions of the original soil, carbon (by wet combustion) (Robertson & Shewan, 1935), nitrogen (by Kjeldahl method) and moisture (drying at 105° C.) were determined. *p*H values were obtained on the moist samples, using the standard quinhydrone electrode.

*Ether-alcohol-water extraction.* The soil, 25 g. in the organic layers, 50–100 g. in the inorganic layers, was extracted for 24–30 hr. in a Soxhlet apparatus with ethyl ether, the ether-soluble material dried for ½ hr. in an oven at 100° C., and weighed on cooling. The soil was transferred to a 500 c.c. flask, 100–150 c.c. alcohol added and boiled on a steam-bath under a reflux for 2 hr. The hot alcohol was filtered off through a Büchner funnel, using a hard filter paper (Whatman No. 50). The soil was thoroughly washed with hot alcohol. The residue, after the evaporation of the alcohol, was dried quickly at 100° C. and weighed.

The ether-alcohol-extracted material was treated with 100–200 c.c. of water and boiled under a reflux for 2 hr., filtered through the paper used in the alcohol extraction (owing to vigorous frothing, boiling with water had to be very carefully regulated). The filtrate on cooling was made up to volume (1 l.), and on one portion, after concentration, nitrogen was determined by Kjeldahl's method. Another portion of the filtrate, in a weighed basin, was evaporated down to dryness on the steam-bath, dried in the oven for ½ hr. and weighed, then ashed and weighed again. The total ash-free water-soluble organic material was calculated for the whole sample. Water extraction was performed only on the organic layers of the profile, owing to the filtration difficulties in the mineral layers.

*Hemicellulose, amide nitrogen, etc.* The soil, from the Büchner funnel, was dried in the oven and weighed. A portion of known weight, usually half of the residual soil, was treated with 300–350 c.c. of 2% hydrochloric acid in a 750 c.c. flask and boiled for 5 hr. under a reflux. After cooling, the solution was filtered and made up to known volume. On aliquots, reducing sugars were estimated according to the modified Lane & Eynon method already described. On another portion, amide nitrogen was determined by neutralizing the acid with milk of lime, adding 5 g. of "heavy" magnesium oxide, and distilling off the ammonia into standard acid, using brom-cresol-purple as an indicator. The total nitrogen was determined on a further aliqnot by Kjeldahl's method. The reducing sugars (hemicellulose), amide nitrogen, and total nitrogen were then calculated for the total original sample.

*Cellulose.* The soil, after hydrolysis with hydrochloric acid, was transferred from the filter paper to a weighed dish by means of a jet of water from a wash-bottle, evaporated to dryness and weighed. (The procedure of Waksman in which the filter paper is weighed before and after filtration was found to be most unsatisfactory, owing to the rapid absorption of moisture by the dried filter paper.) A portion of this residual soil, usually 2 or 3 g. in organic layers, 10–15 g. in mineral soils, was then transferred to a 100 c.c. stoppered conical flask, 25 c.c. of 80% sulphuric acid added, and allowed to stand for 2½ hr. at 12–14° C. The contents were transferred to a litre flask, 875 c.c. of distilled water added and the solution boiled for 5 hr. under a reflux. On cooling and filtering, reducing sugars were estimated as before, and nitrogen, if desired, on aliquots of the filtrate. The cellulose, and nitrogen (in solution), were calculated for the original sample.

*Lignins ("lignin-protein" complex).* The soil from the filter paper was transferred to a tared evaporating dish as before and weighed. On portions carbon (by wet combustion), nitrogen (by Kjeldahl's method) and ash were all determined, and hence, using Waksman's (1930*b*) formula, the lignin-protein complexes were calculated for the original sample.

All results are stated on the basis of total organic matter, i.e. carbon percentage multiplied by 1·724.

## APPLICATION OF THE METHOD TO THE SOIL PROFILES

### *Profile descriptions*

The methods used in sampling the soil profiles were essentially those developed by the Russian workers (Polynov *et al.* 1929), in which a pit is dug to the parent soil material, and samples are taken from the different soil horizons. The $A_0$ horizon was divided where possible into the Hesselman "F" and "H" layers (Hesselman, 1926). The loose litter was composed of newly fallen leaves, since all samples were taken in the late autumn.

*Profile* 1

| | |
|---|---|
| *Locality.* | Fintray, Aberdeenshire. |
| *Site.* | Gentle slope (3–4°) at 300 ft. above sea-level. |
| *Vegetation.* | Beechwood with *Holcus mollis* and *Vaccinium Myrtillus.* |
| *Parent material.* | Boulder clay over micaceous siliceous gneiss. |
| $A_0$ (1) 0–5 cm. | Layer of newly fallen beech leaves. "LL" layer. |
| (2) 5–13 cm. | Light yellow to dark brown mat of partially decomposed beech leaves with numerous rootlets. "F" layer. |
| (3) 13–13·5 cm. | Black well-decomposed organic material, fairly compact. "H" layer. |
| $A_1$ 13·5–15 cm. | Black sandy layer, with numerous roots, open-textured. |
| $B_1$ 15–27 cm. | Brown, sandy in texture and loose in structure, with a few roots and frequent rock fragments, earthworms present. |
| $B_2$ 27–35 cm. | Lighter brown in colour, numerous roots, stony. |
| $B_3$ 35–50 cm. | Darker brown, fairly compact, gritty in texture, scarcely altered boulder clay. |
| $C$ 50–60 cm. | Greyish brown mottled boulder clay, very compact and stony. |

*Profile* 2

| | |
|---|---|
| *Locality.* | Aboyne, Aberdeenshire. |
| *Site.* | Flat terrace above river, 450 ft. above sea-level. |
| *Vegetation.* | Oakwood, with occasional Scots fir, *Pteris aquilina, Hypnum splendens, Deschampsia flexuosa, Oxalis acetosella.* |
| *Parent material.* | Sand over boulder clay. |
| $A_0$ (1) 0–3 cm. | Newly fallen oak leaves. "LL" layer. |
| (2) 3–7 cm. | Compact leaf litter, partially decomposed, light yellow to brown in colour. "F" layer. |

| | | |
|---|---|---|
| $A_0$ (3) | 7–14 cm. | Dark brown, well decomposed leaves, with many rootlets, still showing part of plant structure. "H" layer. |
| $A_1$ | 14–20 cm. | Black humus layer, compact, with much mineral matter, and many roots, earthworms present. |
| $A_2$ | 20–22 cm. | Greyish black, containing much humus and bracken rhizomes—slight podsolization. |
| $B_1$ | 22–35 cm. | Greyish brown layer, containing some humus, fairly compact, with frequent rock fragments. |
| $B_2$ | 35–47 cm. | Brown-black layer, compact. |
| $B_3$ | 47–52 cm. | Reddish brown layer of variable thickness, sandy in texture, passing into: |
| $C$ | 52–75 cm. | Greyish yellow sandy boulder clay. |

*Profile 3*

| | | |
|---|---|---|
| *Locality.* | | Potarch, Aberdeenshire. |
| *Site.* | | Gentle slope towards river bank, 450 ft. above sea-level. |
| *Vegetation.* | | Birchwood, with *Pteris*, *Deschampsia flexuosa*, *Hypnum splendens*. *Oxalis acetosella*, *Veronica montana*, *Rubus Idæus*. |
| *Parent material.* | | Boulder clay over gneiss. |
| $A_0$ | 0–5 cm. | Variable, consisting of newly fallen leaves, with a little of last year's litter—very little of true "F" layer present, mixture of moss, grasses, etc. "LL" and "F" layers. |
| $A_1$ | 5–10 cm. | Brownish black humose layer, open-textured, numerous roots and small stones. |
| $A_2$ | 10–15 cm. | Greyish brown, sandy texture and porous, numerous roots, earthworms present. |
| $B_1$ | 15–23 cm. | Brownish, stony, open-textured, fewer roots. |
| $B_2$ | 23–27 cm. | Reddish brown layer, more compact than the preceding layer, stony. |
| $C$ | 27–35 cm. | Fawn-coloured boulder clay, compact and very stony. |

*Profile 4*

| | | |
|---|---|---|
| *Locality.* | | Dinnet Moor, Aberdeenshire. |
| *Site.* | | Flat heath ground, 600 ft. above sea-level. |
| *Vegetation.* | | *Calluna vulgaris*, with mosses, chiefly *Hypnum splendens*, *Hylocomium* spp. |
| *Parent material.* | | Sand and gravel of glacial origin. |
| $A_0$ (1) | 0–1 cm. | Thin cover of heather litter and moss, slight in extent. "LL" layer. |
| (2) | 1–5 cm. | Dark-coloured layer of organic material with some mineral matter. "F" layer. |
| (3) | 5–10 cm. | Black mineral layer of variable thickness, not very compact, gritty. "H" layer. |
| $A_1$ | 10–20 cm. | Blackish in colour with numerous roots, sandy and open-textured with frequent rock fragments. |
| $A_2$ | 20–23 cm. | Grey to greyish brown layer, sandy in texture, more compac than the previous layer. |

| | | |
|---|---|---|
| $B_1$ | 23–60 cm. | Brownish red layer, sandy with numerous stones, no roots in lower part of the layer. |
| $C$ | 60–70 cm. | Yellow-brown sandy gravel, very open in texture. |

*Profile* 5

| | |
|---|---|
| *Locality.* | Ballochbuie, Aberdeenshire. |
| *Site.* | Bank above river, 1050 ft. above sea-level. |
| *Vegetation.* | Scots pine, *Vaccinium Myrtillus*, and *V. Vitis-idæa*, *Calluna*. |
| *Parent material.* | Granitic glacial sand. |
| $A_0$ (1) 0–1 cm. | Newly fallen needles. "LL" layer. |
| (2) 1–3 cm. | Layer of partially decomposed pine needles, loose and open-textured, rootlets, little or no mineral matter. "F" layer. |
| (3) 3–17 cm. | Brown humified layer, plant structure not clearly visible, friable and loose-textured, containing many dead roots. "H" layer. |
| $A_1$ 17–23 cm. | Black greasy layer very moist and finely textured; compact. |
| $A_2$ 23– ? | White leached layer (not sampled). |

*Profile* 6

| | |
|---|---|
| *Locality.* | Strachan, Aberdeenshire. |
| *Site.* | Gentle slope, 300 ft. above sea-level. |
| *Vegetation.* | Mossy pinewood (*Pinus sylvestris*), *Hylocomium triquetrum*, *H. splendens*, *Hypnum schreberi*, *Calluna vulgaris*, etc. |
| *Parent material.* | Sand and gravel over granite. |
| $A_0$ (1) 0–6 cm. | Newly fallen needles. "LL" layer. |
| (2) ,, | Partially decomposed needle litter. "$LL_2$" layer. |
| (3) ,, | Much decomposed needle litter, loose open texture, little or no mineral matter. "$F_1$" and "$F_2$" layers. |
| $A_1$ 6–10 cm. | Greyish sandy, dry, loose, few stones, much gravel; roots, porous, structureless. |
| $A_2$ 10–30 cm. | Ashy grey, slightly stained in places with humus; fairly compact; dry, friable and apparently free; roots present; few large stones but much gravel. |
| $B_1$ 30–40 cm. | Rusty to coffee brown gravelly sand; fairly well compacted but friable; large stones rare. |
| $B$–$C$ 40–100 cm. | Rusty and grey mottled layer, gravelly sand, large stones occasional, roots penetrate, fairly compact. At foot hard pan, not continuous, and in places represented by staining only. |

Of these six profiles, only Nos. 4, 5 and 6 (under *Calluna* and pine) were strongly podsolized (raw humus types), although the oak profile (mull type) showed slight evidence of podsolization. All the profiles, except the heath and pine types, would probably be included in the "superficial mull" category of the Bornebusch (1933) classification, although perhaps only the birch sample would strictly fall into this type. For comparative purposes, the pine profile investigated by Smolik (1933), according to Waksman's original directions, is given, since this is one

of the few published accounts of the complete analysis of a forest soil profile by the method of proximate analysis.

The proximate analyses of the above profiles 1–6 are given in Tables II–VII and IX–XI. The values for the $A_0$ and $A_1$ layers are the mean of closely agreeing duplicates; in the other layers, single determinations alone were made. The nitrogen fractionation was completed on three profiles only, namely Nos. 1, 3 and 4.

The results may be conveniently discussed from three aspects: (1) the differences in the composition of the leaf litter from the various stands, (2) the changes taking place during its decomposition and the formation of humus, and (3) the subsequent changes in the composition of the organic matter throughout the whole profile.

### (1) *The differences in the composition of the leaf litter*

The most important differences in the composition of the original materials are to be found in (*a*) the ether-alcohol-water soluble materials, (*b*) the cellulose, and (*c*) the lignin and proteins.

(*a*) *The ether-alcohol-water soluble materials.*

Except in birch leaves, which, as might be expected, have an exceptionally high ether-soluble content, the deciduous forest litter is lower in ether-soluble materials as compared with either *Calluna* or pine needles. On the other hand, the alcohol-water-soluble materials are much lower in Dinnet Moor (*Calluna*) than in the others.

Table II. *Proximate analysis. The ether-alcohol-water soluble materials (expressed as a percentage of total organic matter (carbon* × 1·724))

| | Materials soluble in | | |
|---|---|---|---|
| Soil sample | Ether | Alcohol | Water |
| Fintray beech "LL" | 3·5 | 7·0 | 5·4 |
| Aboyne oak "LL" | 3·8 | 7·3 | 12·9 |
| Potarch birch "LL" | 10·3 | 6·1 | 6·7 |
| Dinnet Moor "LL" | 5·7 | 4·2 | 2·9 |
| Ballochbuie pine "LL" | 11·5 | 4·2 | — |
| Strachan pine "LL" | 10·5 | 5·4 | 7·4 |
| Cf. Smolik's pine "LL" | 7·2 | 5·7 | 6·6 |

The appearance of the ether-soluble materials in the loose litter from the various stands shows some interesting differences. From both oak and *Calluna* it is canary yellow in colour and dull in appearance, but whereas the oak is neutral, the *Calluna* is acid to litmus. The ether extracts from the remaining samples are all dark brown in colour and acid to litmus. That from pine needles is shiny and resinous in appearance,

and very acid. The major portion of this extract is neutral to litmus, with a melting-point of 77° C. The remaining portion is very acid, semi-solid and sticky and is probably a resinous acid.

On the other hand, the alcohol- and water-soluble materials, in every case, are dark coloured and acid to litmus. From nearly every sample, the boiling alcohol extract deposits on cooling a neutral wax.

(b) *Hemicellulose and cellulose.*

Whilst the hemicellulose is fairly similar in amount in all the samples studied, the cellulose varies considerably. From Table III, it will be seen that *Calluna* (Dinnet Moor) is outstanding in having a low cellulose content (10·4 %); the percentage rises in the beech (13·3 %), birch (13·9 %) and oak (23·0 %), and is highest in the pine (32·7 %) where it is nearly three times that of *Calluna.*

(c) *Lignin and protein.*

Lignin, like the hemicellulose, is fairly constant in amount in all samples, but the protein is usually higher in the deciduous litter than in either the pine needles or *Calluna.*

Table III. *Hemicellulose, cellulose, lignin and protein (expressed as a percentage of total organic matter)*

| Soil sample | Hemicellulose | Cellulose | Lignin | Protein |
|---|---|---|---|---|
| Fintray beech "LL" | 17·9 | 13·3 | 33·4 | 11·8 |
| Aboyne oak "LL" | 22·2 | 23·0 | 32·0 | 10·2 |
| Potarch birch "LL" | 18·0 | 13·9 | 37·9 | 8·4 |
| Dinnet Moor "LL" | 21·4 | 10·4 | 39·6 | 7·9 |
| Ballochbuie pine "LL" | 10·3 | 32·7 | — | 4·1 |
| Strachan pine "LL" | 15·6 | 25·0 | — | 7·6 |
| Cf. Smolik's pine "LL" | 17·1 | 13·8 | 40·8 | 7·2 |

From Table IV it may be observed that the *p*H and nitrogen values are high in the deciduous litter, in accordance with Hesselmann's findings. The C/N ratios vary within the wide limits of 30–87; these will be discussed more fully later.

Table IV. *Proximate analysis (pH values, C/N ratios and ash)*

| Soil samples | *p*H moist | Carbon | Nitrogen | C/N ratio | Total organic matter C × 1·724 | Ash |
|---|---|---|---|---|---|---|
| Fintray beech "LL" | 5·45 | 50·0 | 1·63 | 30·7 | 86·2 | 3·7 |
| Aboyne oak "LL" | 4·92 | 50·0 | 1·40 | 35·7 | 86·1 | 5·3 |
| Potarch birch "LL" | 5·32 | 51·6 | 1·20 | 43·0 | 89·0 | 2·9 |
| Dinnet Moor "LL" | 4·22 | 49·3 | 1·22 | 40·5 | 84·9 | 8·0 |
| Ballochbuie pine "LL" | 4·20 | 51·4 | 0·59 | 87·4 | 88·5 | 3·0 |
| Strachan pine "LL" | — | 48·9 | 1·02 | 47·9 | 84·3 | — |

The organic matter and ash percentages should total 100, but in no case does this actually occur. In both the "LL" and the "F" layers the factor obtained by dividing the loss on ignition (which should be a fairly accurate measure of the total organic matter in the "LL" and "F" layers) by the carbon percentage was found to vary within the limits 1·79–2·03 with an average value of 1·92. It is possible, therefore, that for such the usual factor 1·724 may be too low.

In all the samples in which nitrogen complexes have been fractionated, it will be seen (Table V) that a large portion (40–55 % of the total nitrogen in the original sample) is rendered soluble by the 2 % HCl treatment, 5–7 % of which is amide nitrogen. Of the remaining nitrogen a portion is hydrolysed by concentrated sulphuric acid, but the greater part of it appears to be bound to the lignin complex or to be unacted on by the acid treatments.

Table V. *Nitrogen fractionation (expressed as percentage of total nitrogen)*

| Soil sample | Nitrogen soluble by 2 % HCl: Non-amide | Amide | Total | Water soluble nitrogen | Nitrogen soluble by 80 % $H_2SO_4$ (by difference) | Lignin nitrogen |
|---|---|---|---|---|---|---|
| Fintray beech "LL" | 39·5 | 5·2 | 44·7 | 3·6 | 20·4 (11·2)* | 31·2 |
| Potarch birch "LL" | 33·5 | 6·4 | 39·9 | 5·9 | 21·8 (15·4)* | 32·3 |
| Dinnet Moor "LL" | 47·1 | 7·6 | 54·6 | — | 2·2 | 43·1 |
| Strachan pine "LL" | 27·2 | 5·2 | 32·4 | — | — | — |

* *Experimental values.*

### (2) *The decomposition of these materials in the soil*

When plant materials are introduced into the soil they undergo immediate decomposition provided the temperature and moisture conditions are favourable. The actual process of decomposition is, of course, extremely complicated, its rate and character depending upon many factors, climatic, physical, chemical, and biological. Granted favourable decomposition conditions, however, it will be seen from Tables VI and VII that the plant materials do not decompose as a whole, but that particular constituents tend to disappear or accumulate more readily than others.

Comparing the decomposition layers (i.e. the "F", "H" layers) of the various profiles with the data for the loose litter already given, Tables II–V, the following facts emerge. In general the ether-alcohol-water soluble materials decrease in all profiles. The hemicellulose decreases only slightly, and in *Calluna* heath and pine (Ballochbuie) it actually increases in the "H" layer.

Table VI. *Proximate analysis. The ether-alcohol-water soluble materials (expressed as a percentage of total organic matter (carbon × 1·724))*

| Soil sample | | Materials soluble in: Ether | Alcohol | Water |
|---|---|---|---|---|
| Fintray beech | "F" | 1·9 | 7·0 | 5·4 |
| | "H" | 2·5 | 3·4 | 7·2 |
| Aboyne oak | "F" | 2·8 | 5·0 | 8·4 |
| | "H" | 2·2 | 3·5 | 6·3 |
| Potarch birch | "F" & "H" | 3·5 | 3·1 | 6·5 |
| Dinnet Moor | "F" | 5·7 | 3·9 | 3·3 |
| | "H" | 4·1 | 4·6 | — |
| Ballochbuie pine | "F" | 6·4 | 3·0 | — |
| | "H" | 2·8 | 4·7 | 6·4 |
| Strachan pine | "F" | 5·6 | 4·8 | 3·9 |
| Cf. Smolik's pine | "F" | 4·3 | 5·2 | 5·7 |
| | "H" | 4·1 | 4·2 | 5·3 |

Table VII. *Proximate analysis. Hemicellulose, cellulose, lignin and protein (expressed as a percentage of total organic matter (carbon × 1·724))*

| Soil sample | | Hemicellulose | Cellulose | Lignin | Protein |
|---|---|---|---|---|---|
| Fintray beech | "F" | 18·8 | 7·7 | 37·8 | 14·9 |
| | "H" | 10·4 | Trace | 38·4 | 20·2 |
| Aboyne oak | "F" | 17·6 | 16·6 | — | 15·8 |
| | "H" | 16·6 | 15·3 | 40·0 | 18·1 |
| Potarch birch | "F" & "H" | 16·6 | 9·5 | 41·8 | 14·7 |
| Dinnet Moor | "F" | 21·3 | 8·0 | 47·6 | 9·2 |
| | "H" | 26·1 | 9·4 | 39·3 | 12·0 |
| Ballochbuie pine | "F" | 7·8 | 13·5 | — | 8·9 |
| | "H" | 11·6 | 3·0 | — | 10·3 |
| Strachan pine | "$F_1$" | 15·8 | 10·5 | — | 11·7 |
| Cf. Smolik's pine | "F" | 12·3 | 10·3 | 49·6 | 11·3 |
| | "H" | 11·6 | 5·2 | 55·7 | 11·4 |

The samples in which uronic acids were estimated show that these compounds do not disappear through decomposition.

Table VIII. *The hemicellulose content of various soil horizons (expressed as a percentage of carbon × 1·724)*

| Material | Pentosans | % HCl hydrolysis | Uronic acid |
|---|---|---|---|
| Pine needles ("$LL_1$") | 4·75 | 11·00 | 7·16 |
| ,, "F" | 4·41 | 8·28 | 8·50 |
| Beech "$LL_1$" | — | 22·15 | 10·03 |
| ,, $A_1$ | — | 11·10 | 12·00 |
| *Calluna* heath "F" | — | 21·30 | 5·72 |
| ,, $B_1$ | — | 2·0 | 38·5 |

It is known that certain polyuronides are attacked by micro-organisms (Waksman & Allen, 1934), but whether the persistence of

these uronic acids is due to the formation of complexes or to actual microbiological synthesis is, at present, impossible to say. Another point of interest is the exceptionally high uronic acid content obtained in the *B* layer of the *Calluna* heath (over 30%). Waksman found a similar percentage in the *B* layer of a spruce profile. Tiurin & Kononova (1934), after hydrolysing the hemicellulose with 2% HCl, estimated the uronic acid content in the iron precipitate obtained prior to sugar determination, and found a large percentage there (over 50% of the total), i.e. the uronic acids are definitely adsorbed by the iron-aluminium precipitate. The leaching of the iron in combination with the uronic acids, and accumulation in the *B* layer, may account for the large percentage of polyuronides in this particular soil horizon. It is probable, however, that the carbon dioxide by which the uronic acids are determined may originate from other organic compounds, and the whole problem of the accurate determination of polyuronides in soils requires careful investigation.

In contrast to the hemicellulose, the cellulose generally decreases rapidly; whilst there is an increase in the lignins or modified lignin complexes and in the proteins.

It is obvious, therefore, that irrespective of the differences in the composition of the original materials, by the process of humification there results in the soil an organic complex, acid in nature, consisting chiefly of lignin or modified lignin complexes, protein and hemicellulose, with little or no cellulose and a small percentage of ether-alcohol soluble materials. These results conform more or less to the present-day views concerning the chemistry of humus, as expressed by Grosskopf, Waksman and others.

### (3) *Changes in composition throughout the whole profile*

The most interesting feature of this investigation is the difference in the composition of the organic matter in the lower layers of the profile. In Tables IX–XI are given the proximate analysis of the lower profile layers. It will be seen that whilst differences between the individual profiles are small, those between the two humus types, raw humus and mull, are quite appreciable. The differences, it will be noted, occur mainly in the hemicellulose and lignin.

In the raw humus, the amount of hemicellulose falls abruptly from the $A_1$ to the $A_2$ layer, and below the latter it is entirely absent or nearly so. In the mull type, on the other hand, the hemicellulose persists throughout the whole profile, there being a slight decrease in the $A_1$

layer only. In the heath profile, as in Smolik's pine profile, the lignin-protein complexes increase continuously down the profile, whilst in the others, after an initial increase in the $A_1$ layer the lignin percentage actually decreases.

Table IX. *Proximate analysis (expressed as a percentage of total organic matter (i.e. carbon* × 1·724))

| Soil sample | | Materials soluble in Ether | Materials soluble in Alcohol | Hemi-celluloses | Cellu-loses | Lignin | Protein |
|---|---|---|---|---|---|---|---|
| Fintray beech | $A_1$ | 2·8 | 5·0 | 6·2 | 0 | 45·0 | 23·6 |
| | $B_1$ | 1·8 | 4·9 | 15·8 | 0 | 41·3 | 25·8 |
| | $B_2$ | 1·8 | 3·0 | 15·7 | 0 | 41·9 | 24·5 |
| | $B_3$ | 1·6 | 2·1 | 12·2 | 0 | — | 25·3 |
| | $C$ | 6·9 | 3·4 | ? | 0 | — | 44·9 |
| Aboyne oak | $A_1$ | 2·1 | 3·2 | 11·1 | 11·0 | — | 19·0 |
| | $A_2$ | 2·9 | 2·9 | 12·9 | Trace | 42·3 | 21·7 |
| | $B_1$ | 1·6 | 2·0 | 10·0 | — | — | 24·3 |
| | $B_2$ | 1·1 | 2·2 | 7·2 | — | 38·0 | 25·0 |
| | $B_3$ | 0·9 | 1·7 | 5·7 | — | — | 24·9 |
| Potarch birch | $A_1$ | 2·1 | 1·7 | 15·8 | 3·7 | 48·7 | 20·7 |
| | $A_2$ | 2·1 | 1·7 | 13·1 | Trace | 46·2 | 23·1 |
| | $B_1$ | 2·3 | 1·7 | 14·6 | 0 | 42·7 | 24·1 |
| | $B_2$ | 1·6 | 1·4 | 12·0 | 0 | 32·5 | 23·2 |
| Dinnet Moor | $A_1$ | 2·9 | 3·9 | 17·1 | 0 | 30·4 | 14·1 |
| | $A_2$ | 3·1 | 3·0 | 3·3 | 0 | 45·0 | 16·4 |
| | $B_1$ | 4·0 | 2·7 | Trace | 0 | 69·6 | 21·9 |
| | $B_2$ | 2·7 | 1·6 | 0 | 0 | 53·0 | 18·9 |
| | $C$ | 3·4 | 2·2 | 0 | 0 | — | — |
| Strachan pine | $A_1$ | 4·7 | 5·2 | 15·1 | Trace | — | 16·4 |
| | $A_2$ | 4·8 | 10·6 | 11·6 | 0 | — | 39·2 |
| | $B_1$ | — | 7·5 | 0 | 0 | — | 36·5 |
| Cf. Smolik's | $A_1$ | 5·2 | 5·3 | 11·5 | 2·9 | 54·0 | 13·1 |
| pine | $A_2$ | 4·4 | 5·5 | 7·9 | 1·4 | 57·8 | 20·2 |
| profile | $B$ | 1·5 | 3·5 | 3·8 | 1·4 | 38·2 | 41·6 |
| | $C$ | 0·2 | 0·7 | 0 | 0 | — | — |

The fractionation of the nitrogenous compounds by the Waksman procedure is given in Table X.

The total nitrogen and amide nitrogen dissolved by hydrolysis with 2 % HCl generally increases with depth in all the profiles. In the $B$ layer of the *Calluna* heath podsol this total nitrogen shows a marked increase constituting over 63 % of the total nitrogen in the original sample. It is generally assumed with Weis (1929), that the nitrogen in the $B$ horizon is protein in nature, and it is unfortunate that the Waksman method can throw little light on this problem. From the data available, however, these nitrogenous compounds in the accumulation horizon appear to be distinct from those in the layers above or below it ($A_2$ or $C$). The large percentage of the nitrogen bound to the lignin or not hydrolysed by the various acid treatments should also be noted.

Table X. *Fractionation of the nitrogenous compounds (expressed as a percentage of total nitrogen)*

| Soil sample | Nitrogen hydrolysed by 2% HCl | | | Water soluble nitrogen | Nitrogen soluble by 80% $H_2SO_4$ (by difference) | Lignin nitrogen |
|---|---|---|---|---|---|---|
| | Non-amide | Amide | Total | | | |
| Fintray beech "F" | 36·9 | 6·8 | 43·7 | 4·20 | 19·6 (12·6)* | 36·4 |
| "H" | 25·1 | 9·6 | 36·7 | — | 37·0 (19·4) | 26·3 |
| $A_1$ | 33·9 | 8·6 | 42·5 | — | 5·9 (11·7) | 31·6 |
| $B_1$ | 42·1 | 12·2 | 54·3 | — | 27·0 (20·4) | 18·8 |
| $B_2$ | 47·6 | 13·8 | 61·4 | — | 14·4 — | 24·2 |
| $B_3$ | 43·8 | 15·1 | 58·9 | — | — — | — |
| $C$ | 82·5 | 8·1 | 90·6 | — | — — | — |
| Potarch birch "F" & "H" | 31·8 | 8·1 | 39·9 | — | 25·8 (14·6) | 28·6 |
| $A_1$ | 35·4 | 13·4 | 48·8 | — | 10·6 | 40·6 |
| $A_2$ | 38·2 | 14·3 | 52·4 | — | 10·9 | 36·7 |
| $B_1$ | 38·0 | 14·8 | 52·8 | — | 10·4 | 36·8 |
| $B_2$ | 40·3 | 13·8 | 54·1 | — | 9·2 | 36·6 |
| Dinnet Moor "F" | 35·5 | 9·6 | 45·1 | — | 17·5 | 37·4 |
| "H" | 44·4 | 10·7 | 55·1 | — | 5·7 | 39·2 |
| $A_1$ | 43·8 | 13·3 | 57·0 | — | 5·0 | 37·9 |
| $A_2$ | 18·8 | 31·8 | 50·7 | — | 0 | 50·0 |
| $B_1$ | 42·6 | 20·4 | 62·9 | — | 16·0 | 21·1 |
| $B_2$ | 24·8 | 20·8 | 45·6 | — | 12·0 | 42·4 |
| Strachan pine "$F_1$" | 30·3 | 8·1 | 38·4 | — | — | — |
| $A_1$ | 37·3 | 14·0 | 51·3 | — | — | — |
| $A_2$ | 50·8 | 11·7 | 62·5 | — | — | — |
| $B_1$ | 49·1 | 13·0 | 52·2 | — | — | — |

* The figures in brackets are the actual experimental values.

It is still asserted in most soil science text-books (Robinson, 1932) that C/N ratios vary within narrow limits and are generally found to be almost the value of 10. The work of Leighty & Shorey (1930), in which nearly 200 soils were examined, has shown that the ratio may vary within the wide limits of 3·5–35·2. Much indeed has been written upon C/N ratios (more particularly with regard to agricultural soils) (Russell, 1932), but so far as the above five profiles are concerned, all that can be said is that they generally decrease down the profile, and in no case does the ratio equal 10 (see Table XI).

In summing up the results generally, it will be seen that the profiles can be divided into two groups: (1) Fintray—beech, Aboyne—oak, and Potarch—birch; and (2) Dinnet Moor—*Calluna* and Strachan—pine. The first group corresponds to the mull type, in which the hemicellulose appears to persist in the lower profile layers, whilst the lignins tend to decrease. In the second group, the raw humus type, the hemicellulose fraction disappears and the lignins increase. It is of interest in this connexion to note the parallel between the above data concerning mull and raw humus, and the biological results of Falck (1926). According

Table XI. *pH's, nitrogen, C/N ratios, etc.*

| Soil sample | | *p*H (moist) | Carbon as % oven-dry soil | Nitrogen as % oven-dry soil | C/N ratio |
|---|---|---|---|---|---|
| Fintray beech | "F" | 4·34 | 47·21 | 1·936 | 24·4 |
| | "H" | 3·42 | 25·00 | 1·391 | 18·0 |
| | $A_1$ | 3·78 | 15·43 | 1·003 | 15·4 |
| | $B_1$ | 3·73 | 7·18 | 0·511 | 14·1 |
| | $B_2$ | 4·06 | 4·02 | 0·272 | 14·8 |
| | $B_3$ | 4·03 | 2·91 | 0·203 | 14·3 |
| | $C$ | 4·48 | 0·19 | 0·024 | 7·9 |
| Aboyne oak | "F" | 4·50 | 49·06 | 2·144 | 22·9 |
| | "H" | 4·82 | 43·05 | 2·154 | 20·0 |
| | $A_1$ | 4·23 | 16·06 | 0·841 | 19·1 |
| | $A_2$ | 4·37 | 11·14 | 0·650 | 17·1 |
| | $B_1$ | 4·62 | 6·40 | 0·430 | 14·9 |
| | $B_2$ | 4·66 | 5·24 | 0·362 | 14·5 |
| | $B_3$ | 4·71 | 3·89 | 0·264 | 14·8 |
| Potarch birch | "F" | 4·85 | 34·63 | 1·401 | 24·7 |
| | $A_1$ | 5·29 | 7·31 | 0·416 | 17·6 |
| | $A_2$ | 5·53 | 5·53 | 0·351 | 15·8 |
| | $B_1$ | 5·48 | 4·85 | 0·323 | 15·0 |
| | $B_2$ | 5·40 | 3·21 | 0·206 | 15·6 |
| Dinnet Moor | "F" | 3·96 | 43·30 | 1·242 | 34·9 |
| | "H" | 3·86 | 18·11 | 0·650 | 27·9 |
| | $A_1$ | 4·33 | 4·74 | 0·193 | 24·5 |
| | $A_2$ | 4·58 | 1·29 | 0·059 | 21·9 |
| | $B_1$ | 5·10 | 2·34 | 0·143 | 16·4 |
| | $B_2$ | 5·42 | 1·36 | 0·073 | 18·1 |
| | $C$ | 5·31 | 0·74 | 0·024 | 30·8 |
| Ballochbuie pine | "F" | 3·96 | 54·43 | 1·341 | 40·6 |
| | "H" | 3·70 | 52·65 | 1·498 | 35·1 |
| | $A_1$ | 3·96 | 48·67 | 1·936 | 25·1 |

to this author, the biological decomposition of "streu" (loose litter) may proceed in two directions: (1) "corrosion", in which both cellulose and lignin are destroyed by fungi; and (2) "destruction", in which lignin accumulates, whilst cellulose disappears. The first process is said to occur in mull soils, the second in raw humus soil (Waksman, 1931). In the raw humus samples analysed, the accumulation of lignin and disappearance of cellulose is probably similar to Falck's "destruction" phenomenon, whilst in the mull soils, more particularly in the birch sample, both lignin and cellulose tend to disappear, i.e. the direction of decomposition corresponds to Falck's "corrosion".

## SUMMARY

1. The proximate analysis has been made of several profiles from the north-east of Scotland, these including Scots pine, beech, birch and *Calluna* heath.

2. The system of proximate analysis proposed by Waksman has been used as a basis for the investigation, but various modifications have been

*introduced, e.g. sugar and cellulose determinations*, with a view to obtaining greater accuracy.

3. Although a restricted number of profiles has been investigated, the results indicate that these may be divided into two groups, viz. raw humus and mull types, according to the manner in which the various fractions have been decomposed.

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# ON THE INFLUENCE OF PROTEIN ON THE FATTENING OF FOWLS

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In the fattening of fowls by the Sussex method a mixture of skim milk and Sussex ground oats is usually employed. Formerly the poultry fattener obtained his liquid skim milk at low prices from neighbouring farms, but with the expansion of the poultry-fattening industry the increasing difficulty in obtaining adequate supplies of skim milk caused many of the poultry fatteners to use dried skim milk as an alternative. In 1931 the writer's attention was drawn to the desire of the industry for cheaper fattening mixtures, and it was decided that the first step in achieving economy would be to ascertain to what extent the level of dried skim milk in the fattening mixture could be reduced without the mixture losing its efficiency. From a theoretical point of view, too, the problem was of interest, since if the fat-producing efficiency of a food were materially affected by the amounts of protein fed with it the net energy value of a food for fat production would vary according to the extent of the protein fed with it.

## Experimental details

Thirty-six Light Sussex-Rhode Island Red cross-cockerels, hatched on 16 April 1930, and reared in Sussex arks on grass range were brought to the laboratory on 12 January 1931 and fed on 2 oz. per head per day of pellets containing dried milk and Sussex ground oats for 7 days. The object of this preliminary period of feeding was to accustom the birds to the use of pellets and to reduce them to a "store" condition. On the morning of 19 January the birds were weighed in a fasting state and divided into three lots of twelve, as near as possible on an average weight basis. The birds in Group A were killed, plucked and placed in cold store at $-10^\circ$ C. Groups B and C were divided into even groups of four and housed in fattening cages. Group B received, per head per day 140 g. of pellets consisting of 80 % S.G.O. and 20 % dried skim milk, and Group B received a similar amount of pellets consisting of 95 % S.G.O.

and 5 % dried skim milk. After 16 days' fattening both groups were killed, plucked, and placed in cold store at $-10^\circ$ C. Bird 81 in Group B refused food on the last day of the experiment and was withdrawn.

## Analytical procedure

On withdrawal from cold store for analysis, the bird was allowed to thaw overnight, and gutted in the usual manner. The gut contents were removed and discarded, and the flesh removed from the bones. The entire bones, and 1000 g. of the minced flesh and offal were dried separately for analysis. The chemical composition of the analytical samples was obtained by the usual methods.

The caloric content of the food mixtures was calculated by using the usual factors of 9·1 calories per gram of ether extract, 4·1 for protein, and 4·1 for carbohydrate.

## Essential data

*Analysis of pellets*

| | Group B<br>80 % S.G.O.<br>20 % dried milk | Group C<br>95 % S.G.O.<br>5 % dried milk |
|---|---|---|
| Moisture | 9·05 | 7·46 |
| Ether extract | 5·10 | 6·05 |
| Protein | 16·14 | 12·47 |
| N-free extract | 58·01 | 60·29 |
| Fibre | 7·01 | 9·25 |
| Ash | 4·69 | 4·48 |

*Weights of birds before and after fattening (in grams)*

| Group A | | Group B | | | Group C | | |
|---|---|---|---|---|---|---|---|
| Bird No. | Weight before fattening | Bird No. | Weight before fattening | Weight after fattening | Bird No. | Weight before fattening | Weight after fattening |
| 69 | 2840 | 67 | 2930 | 3295 | 91 | 3300 | 3610 |
| 66 | 2815 | 99 | 2795 | 3225 | 71 | 2795 | 3060 |
| 74 | 2685 | 79 | 2695 | 3160 | 85 | 2760 | 3115 |
| 92 | 2680 | 77 | 2655 | 3090 | 97 | 2625 | 3055 |
| 95 | 2560 | 80 | 2575 | 3150 | 82 | 2595 | 3050 |
| 100 | 2555 | 73 | 2545 | 3055 | 96 | 2545 | 2925 |
| 86 | 2475 | 76 | 2505 | 2905 | 78 | 2530 | 2970 |
| 65 | 2430 | 98 | 2470 | 2955 | 94 | 2410 | 2800 |
| 87 | 2365 | 93 | 2365 | 2770 | 83 | 2385 | 2735 |
| 68 | 2320 | 90 | 2315 | 2840 | 72 | 2295 | 2715 |
| 75 | 2210 | 81 | Withdrawn | | 88 | 2150 | 2470 |
| 84 | 1755 | 89 | 1855 | 2300 | 70 | 2060 | 2585 |
| Average | 2474 | | 2519 | 2977 | | 2537 | 2924 |

*Total carcase composition (in grams)*

| Group | Total weight | Moisture | Fat | Protein | Ash |
|---|---|---|---|---|---|
| A | 25962 | 17720·5 | 1017·6 | 5787·4 | 1402·0 |
| B | 28932 | 18601·9 | 2909·1 | 5984·8 | 1393·4 |
| C | 31168 | 19891·4 | 3266·7 | 6423·1 | 1555·6 |
| *Average carcase composition* | | | | | |
| A | 2163·5 | 1476·7 | 84·8 | 482·3 | 116·8 |
| B | 2630·2 | 1691·1 | 264·5 | 544·1 | 126·7 |
| C | 2597·1 | 1657·6 | 272·2 | 534·4 | 129·6 |
| *Average carcase composition of Groups B and C at commencement of fattening, based on composition of Group A* | | | | | |
| B | 2186·9 | 1492·7 | 85·7 | 487·5 | 118·1 |
| C | 2203·4 | 1504·0 | 86·4 | 491·2 | 119·0 |
| *Average increases during fattening period* | | | | | |
| B | 443·3 | 198·4 | 178·8 | 56·6 | 8·6 |
| C | 393·7 | 153·6 | 185·8 | 43·2 | 10·6 |

*Energy content of increase in calories*

Group B 1988·3 Group C 1982·8

*Energy content of food intake in calories*

| | Food intake | Calories |
|---|---|---|
| Group B average | 2240 g. | 7871 |
| Group C average | 2240 g. | 7943 |

*Ratio of calories in body increase to food calories*

Group B = 0·252 Group C = 0·249

The caloric content of the body increase was calculated by using the factors 9·43 for every gram of fat and 5·34 for every gram of protein. The figure 9·43 calories was the average calorific value of several samples of poultry fat as determined by the bomb calorimeter, and the value 5·34 calories per gram of protein was extracted from a series of analyses of dried poultry flesh, the gross energy values of which had been determined by the bomb calorimeter.

## DISCUSSION

From the tables given above it will be evident that the principal change in body composition that occurs in the normal fattening process is an increase in fat storage, the increase in protein, though positive, being relatively unimportant. It will be further noted that both groups B and C stored almost identical amounts of energy during the fattening period, and that the ratio of the energy content of the body increase to the energy content of the food was again almost identical, i.e. 0·252 and

0·249. It is therefore quite evident that the extra intake of protein as represented by a total protein percentage of 16·14 in the one ration as compared with 12·47 in the other was without influence on the nature and rate of fattening. It is of considerable importance to realize that an increase in the daily consumption of milk protein from 2·396 to 9·184 g. was without effect on the utilization of the total energy present in the food mixture. From the theoretical aspect of fat metabolism, these results indicate that, provided the body is supplied with its normal maintenance requirement of protein, additional supplies of protein do not exert any beneficial or detrimental effect on the fattening capacity of a food given with it. This is important from the point of view of feeding standards, since if such an effect were established, the net energy or the starch equivalent of a food would vary according to the nature and proportions of protein fed with it.

From the practical point of view, the resnlts indicate that a mixture of Sussex ground oats 95 %, dried skim milk 5 % is just as efficacious in fattening as a mixtnre of Sussex ground oats 80 %, dried skim milk 20 %; a fact of considerable importance to poultry fatteners, since it enables them to cheapen the cost of the fattening ration.

In the experiments outlined above, the birds were fattened at an age of approximately 9 months, whereas the fattener normally fattens birds of 14–17 weeks. In addition, it was not possible, owing to the fact that the birds had to be analysed chemically, to ascertain the influence of the two fattening mixtures on the quality of the carcase, a point of considerable commercial importance. Accordingly steps were taken to ascertain whether younger birds would react in a similar manner, and to test the possible effect on quality of carcase by marketing such birds in the usual manner. The results of these experiments, which were carried out in 1931 at Wye, Kent, by the Southern Sub-Committee of the National Poultry Institute scheme (1935), showed that, judged by live-weight increases and prices realized, a mixture of 13 lb. Sussex ground oats and 1 lb. dried skim milk was as efficacious for the fattening of 14-week-old Light Sussex chickens as a mixture of 13 lb. Sussex ground oats and 2 lb. dried skim milk. Fattening experiments carried out on Light Sussex Rhode Island Red cross-chickens by the National Institute of Poultry Husbandry (Macdonald and Kay, 1937) have also shown that a mixture consisting of 5 % dried skim milk, 95 % Sussex ground oats gave as good fattening results, and proved more economical, than a ration of Sussex ground oats mixed with undiluted separated milk or equal parts of separated milk and water.

## Summary

1. The principal change in the composition of adult fowls during fattening is an increase in the fat content of the body, the protein increase being subordinate in amount.

2. Within the limits studied, the efficiency of conversion of food energy to carcase energy is not affected by variation in the protein content of the ration.

3. A food mixture consisting of 5 % dried skim milk of 95 % Sussex ground oats proved as efficient for fattening fowls as a food mixture consisting of 20 % dried skim milk, and 80 % Sussex ground oats.

4. The food mixtures used gave satisfactory results in fattening when used in pellet form in a dry state.



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# OBSERVATIONS ON THE MINERAL METABOLISM OF PULLETS. III

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(With Five Graphs)

THE skeletal tissues of the fowl can supply calcium for egg-shell formation when the available food calcium is insufficient for this purpose. The evidence on this point has been discussed elsewhere (Common, 1932, 1933, 1936*a*; Deobald *et al.* 1936), particularly in Halnan's (1937) recent review on the role of minerals in poultry nutrition. The present paper describes an experiment which was designed (*a*) to measure the extent to which the body has been depleted of calcium in this way when only soft-shelled or shell-less eggs are being produced, and (*b*) to ascertain what effect, if any, such depletion may have on the relative proportions of calcium, phosphorus, magnesium and carbonate present in the skeletal tissues.

## SCHEME OF EXPERIMENT

The experimental birds consisted of eight White Wyandotte pullets, of the same strain, and hatched between 7 April 1935, and 1 May 1935. They had been reared upon similar rations, and were not laying when they were selected for experiment and placed in metabolism cages on 9 December 1935. After a preliminary period of 10 days in the cages, during which the birds received ration L (see Table I) together with some wheat grain, the birds were given the experimental rations and the determinations of their daily calcium and phosphorus balances were begun.

Four of the birds constituted group L and received a ration of low calcium content; the other four birds constituted group H and received a ration of high calcium content. The compositions of the rations, which were in the form of prepared pellets, are given in Table I.

Two birds from each group were removed and killed immediately after laying their first egg during the experiment. For convenience these two subgroups are given the index letters LF and HF, signifying that

they had received the low calcium ration and high calcium ration respectively, but that they had produced only few eggs when killed. It was anticipated that skeletal depletion would be relatively small in these birds, and that the losses of calcium in eggs would be offset to a greater or less extent by prelaying storage of calcium. It will be seen later from Table IX that these anticipations were fulfilled to a considerable extent.

Table I. *Composition of rations*

| | Ration L (low calcium content) parts by weight | Ration H (high calcium content) parts by weight |
|---|---|---|
| Bran... ... ... | 15 | 15 |
| Pollards ... ... | 20 | 20 |
| Sussex ground oats... | 5 | 5 |
| Extracted soya bean meal ... ... | 5 | 5 |
| Fish meal ... ... | 2·5 | 2·5 |
| Yellow maize meal... | 25·5 | 25·5 |
| Wheat meal... ... | 25 | 25 |
| Cod-liver oil ... | 1 | 1 |
| Sodium chloride ... | 1 | 1 |
| Calcium carbonate... | — | 5 |
| Total | *100* parts | *105* parts |

The remaining two birds in group L were killed when they had begun to lay very soft or almost shell-less eggs, i.e. when skeletal depletion might be regarded as advanced. The two birds of this subgroup were given the index letters LM, signifying that they received the low calcium ration and were killed when they had laid many eggs. The remaining two birds in group H were killed when they had laid in the one case nineteen and in the other seventeen eggs and were still in full lay; they were given the index letters HM, signifying that they had received ration H and laid many eggs.

The carcasses were analysed immediately after the birds were killed.

## Experimental technique

The management and feeding of the birds were conducted as described elsewhere (Common, 1936*a*), except that acid treatment of the droppings was omitted since nitrogen metabolism was not under investigation.

The greatest difficulty was encountered in preventing egg-eating by the birds on the low calcium ration, despite the fact that specially designed battery cages were used. Soft eggs did not roll away quickly enough when laid or were broken in laying, and the birds promptly attacked the

shells. The only satisfactory solution of this problem appears to be constant surveillance, and the removal of eggs immediately they are laid. Such eggs as were secured entire from pullets LM 1 and LM 4 in the present experiment were secured in this way. Fortunately, egg-eating does not vitiate the gross balance figures, but where eggs are even broken it is no longer possible to obtain their fresh weight accurately and so calculate their percentage composition.

## ANALYTICAL METHODS

Calcium and phosphorus determinations were made on the rations, droppings and eggs by methods previously described (Common, 1936*a*).

The analytical data for the rations are given in Table II. The standard deviations cover both analytical and sampling errors, since, in all, twelve samples were drawn at random from different parts of the main bulk before, during and after the experimental period. Analyses made for the express purpose showed that the composition of the pellets did not alter during the course of the experiment.

Table II. *Calcium and phosphorus contents of experimental rations*

| | CaO | | $P_2O_5$ | |
|---|---|---|---|---|
| | % | Standard deviation | % | Standard deviation |
| Ration L | 0·364 | 0·0050 | 1·228 | 0·0067 |
| Ration H | 2·964 | 0·0566 | 1·172 | 0·0176 |

The birds were killed by chloroform. They were then carefully weighed, bled through the external jugular vein and then plucked. The blood and feathers were weighed, and the carcasses dissected as rapidly as possible into the following portions:

(*a*) Oviduct.

(*b*) Ovary.

(*c*) Other viscera except the alimentary canal.

(*d*) Alimentary canal including gizzard and crop.

(*e*) Skin, including comb, preen gland and skin of shanks and feet.

(*f*) Fatty tissue, including as much subcutaneous fat as possible.

(*g*) Muscle, including as much muscular tissue as could be conveniently dissected off the carcass.

(*h*) The tibiae, femora, humeri and sternum, carefully freed from muscle.

(*i*) The residual bony framework with its adhering flesh, the central nervous system and the eyes.

After each portion had been dissected out it was weighed, finely minced and sampled for analysis, except in the case of the last two portions. The long bones and sternum were placed in alcohol after weighing, and reserved for separate treatment. The residual bony framework was placed in boiling distilled water for some minutes to coagulate the flesh, and the bones were then carefully cleaned with a small blunt scalpel. The separated flesh, tendon, etc., were added to the boiling liquor, evaporated and analysed. The bones were extracted with absolute alcohol, and then with ether until fat-free. They were then dried to constant weight, finely ground in a coffee mill and bottled for subsequent analysis. The long bones and sternum were separately prepared in the same fashion.

The analyses of the various portions of the carcass were made by the usual methods: moisture by loss at 105° C.; crude protein by macro-Kjeldahl (factor 6·25); fat by ether extraction of a sample dried in a vacuum oven; crude ash by incineration.

The analyses on the powdered bones were made on samples dried to constant weight to expel moisture picked up again during the process of grinding. Total ash was determined by incineration in an electric muffle at 600° C. The ash was dissolved in HCl and aliquots taken for the determination of calcium, phosphorus and magnesium. Calcium was determined by titration of the oxalate with permanganate, the analyses being checked by gravimetric determinations as the oxide. Phosphorus was determined gravimetrically by separation as phosphomolybdate and weighing as $Mg_2P_2O_7$ after reprecipitation with magnesia mixture. Magnesium was determined gravimetrically as $Mg_2P_2O_7$ on a larger aliquot, after removal of calcium as oxalate, but the large excess of calcium rendered it difficult to achieve as high a degree of accuracy in this case as in the case of the calcium and phosphorus determinations.

Carbonate determinations were made directly on the dry powdered fat-free bone by means of the Conway unit (Conway & Byrne, 1933). 50 mg. bone powder were placed in the outer chamber of the unit and moistened with a few droplets of 30 % alcohol. 1 ml. $N/10$ baryta was then run into the central chamber from a 2 ml. micro-burette attached directly to a reservoir guarded by soda lime tubes; 2 ml. phosphoric acid, sp. gr. 1·40, were quickly run into the outer chamber, the lid was slid into position, and the acid brought into contact with the bone. The unit was left overnight, and the excess baryta titrated next morning with $N/10$ oxalic acid, using phenol-thymol phthalein as indicator (Edwards *et al.* 1935). Blanks were always set up at the same time.

When standardizing this method with $N/10$ $Na_2CO_3$ it was found that for some reason 80 % trichloracetic acid gave high and variable results, and accordingly phosphoric acid was used instead. It gave results with standard carbonate solutions which appeared little less accurate than those obtained when using the Conway unit with $N/10$ solutions for ammonia determinations, and as far as could be seen by the binocular microscope all mineral matter was dissolved from bone by the acid overnight. Replicate determinations seldom disagreed by more than 1/50 ml., and at least four replicates were made of each determination. When using this method it is desirable to work in a well-ventilated laboratory in which no gas burners are burning, and to avoid breathing on the units or burette tip while delivering baryta or on the unit while titrating, precautions common to all baryta titrations.

## EXPERIMENTAL RESULTS, PART I

### *The availability of body calcium reserves for egg-shell formation*

Table III summarizes the progress of the experiment and presents the initial and final live weights of the birds. It will be noted that the laying period appears to be very long in the case of pullet LM 1; the explanation is that this pullet laid shortly after the beginning of the experiment, and intermittently for a few days thereafter; laying then ceased and the bird was not killed until laying had been in progress for a second period. LM 1 is thus not entirely comparable with LM 4 owing to the long intermediate period of calcium storage.

Table III

| No. of bird | Ration | Duration of experiment, days | Day of experiment on which first egg was laid | No. of eggs produced | Initial live weight kg. | Final live weight kg. |
|---|---|---|---|---|---|---|
| LF 2 | Low calcium | 17 | 17 | 2* | 2·50 | 2·95 |
| LF 3 | ,, | 23 | 23 | 1 | 2·56 | 2·93 |
| LM 1 | Low calcium | 73 | 4 | 13 | 2·59 | 2·86 |
| LM 4 | ,, | 48 | 8 | 11 | 2·53 | 2·36 |
| HF 5 | High calcium | 18 | 18 | 1 | 2·52 | 2·76 |
| HF 8 | ,, | 20 | 20 | 1 | 2·28 | 2·74 |
| HM 6 | High calcium | 42 | 17 | 18† | 2·05 | 2·19 |
| HM 7 | ,, | 36 | 10 | 19 | 2·04 | 2·27 |

* The second egg was recovered from the oviduct during dissection.
† The eighteenth egg was recovered from the oviduct during dissection.

Table IV. *Losses in weight during dissection of the carcasses*

| No. of bird | Weight of carcass less feathers and gut contents g. | Total weight of portions of carcass after dissection g. | Loss during dissection due to evaporation g. | Loss during dissection due to evaporation: As percentage of carcass weight less feathers and gut contents |
|---|---|---|---|---|
| LF 2 | 2743·7 | 2558·8 | 184·9 | 6·74 |
| LF 3 | 2709·9 | 2591·4 | 118·5 | 4·37 |
| LM 1 | 2647·4 | 2514·7 | 132·7 | 5·01 |
| LM 4 | 2184·5 | 2047·5 | 137·0 | 6·27 |
| HF 5 | 2506·9 | 2348·3 | 158·6 | 6·33 |
| HF 8 | 2512·5 | 2369·7 | 142·8 | 5·68 |
| HM 6 | 1918·3 | 1820·9 | 97·4 | 5·08 |
| HM 7 | 2076·4 | 1956·9 | 119·5 | 5·76 |

Table V. *Composition of carcasses*

(Based on the total weight of the portions of carcass after dissection.)

| No. of bird | Total weight g. | Weight of fat g. | Fat as percentage of total weight | Fat-free weight g. | CaO g. | Percentage composition on fat-free basis: Moisture | Crude protein | Crude ash | CaO |
|---|---|---|---|---|---|---|---|---|---|
| LF 2 | 2558·8 | 754·7 | 29·49 | 1804·1 | 29·89 | 72·73 | 22·32 | 4·22 | 1·657 |
| LF 3 | 2591·4 | 799·7 | 30·86 | 1791·7 | 34·32 | 72·64 | 21·93 | 4·92 | 1·915 |
| LM 1 | 2514·7 | 718·0 | 28·55 | 1796·7 | 29·11 | 72·90 | 22·94 | 4·15 | 1·620 |
| LM 4 | 2047·5 | 660·4 | 32·25 | 1387·1 | 22·75 | 72·61 | 23·61 | 4·28 | 1·640 |
| HF 5 | 2348·3 | 606·1 | 25·81 | 1742·2 | 38·74 | 71·50 | 23·30 | 5·23 | 2·224 |
| HF 8 | 2369·7 | 487·0 | 20·55 | 1882·7 | 40·05 | 72·21 | 22·47 | 5·03 | 2·127 |
| HM 6 | 1820·9 | 399·8 | 21·96 | 1421·1 | 22·16 | 73·04 | 22·31 | 4·05 | 1·559 |
| HM 7 | 1956·9 | 422·8 | 21·61 | 1534·1 | 24·41 | 74·37 | 21·61 | 4·05 | 1·591 |

Table VI. *Composition of carcasses*

(Based on plucked empty weight and allowing for losses during dissection due to evaporation.)

| No. of bird | Fat free weight of plucked empty carcass g. | Percentage composition on fat-free basis: Moisture | Protein | Ash | CaO |
|---|---|---|---|---|---|
| LF 2 | 1989·0 | 75·26 | 20·24 | 3·83 | 1·503 |
| LF 3 | 1910·2 | 74·34 | 20·57 | 4·63 | 1·797 |
| LM 1 | 1929·4 | 74·76 | 21·37 | 3·87 | 1·509 |
| LM 4 | 1524·1 | 75·07 | 21·49 | 3·89 | 1·493 |
| HF 5 | 1900·8 | 73·88 | 21·35 | 4·80 | 2·038 |
| HF 8 | 2025·5 | 74·17 | 20·88 | 4·68 | 1·977 |
| HM 6 | 1518·5 | 74·77 | 20·88 | 3·79 | 1·459 |
| HM 7 | 1653·6 | 76·22 | 20·05 | 3·75 | 1·476 |

The calculations of the composition of the carcasses from the analytical data were complicated by the losses in weight which occurred during dissection. The relevant data are given in Table IV, from which it will be seen that dissection was accompanied by a loss of between 5·0 and 6·7 % of the empty plucked carcass weight. By far the greater part of such losses is due to evaporation of water during dissection, and the percentage

losses tended to be constant; the higher losses in the cases of LF 2 and HF 5, the first birds dissected, were explicable on account of the somewhat longer time required for these first dissections.

Since the various portions were weighed immediately they were dissected out, and at once quickly minced and subsampled for analysis, the carcass compositions are only strictly valid when the sum of the weights of the various portions is taken as the carcass weight. The compositions calculated in this way are set out in Table V.

If the losses during dissection are taken as being due to moisture losses, it is possible to recalculate the carcass compositions on the empty plucked weight. The compositions calculated in this way are set out in Table VI. Since the percentage losses were approximately constant, the variations in carcass composition as between the different birds follow the same trends whichever table is considered. For the purpose of the present paper the carcass compositions as stated in Table V will be used exclusively, while the data as stated in Table VI are reported for the purposes of comparison with other carcass analyses of poultry. Comparison with the data of Buckner & Martin (1920) is complicated by the fact that these investigators rejected certain portions of the carcasses before analysis.

The first striking feature of Table V is the enormous proportion of fat in the pullets, which amounts to as much as a third of the plucked empty carcass weight in the case of LM 4.

Cruickshank (1934) has also pointed out the large proportion of fat which may be present in the hen. The total fat content was distinctly lower in the case of the birds of group H, suggesting a relationship between calcium metabolism and fat metabolism in the pullet. The effect of the rations on body fat was clearly much greater than any effect due to laying activity; it cannot be said from the data that laying decreased fat content during this experiment.

Table V shows that HF 5 and HF 8 had the highest crude-ash contents expressed on the fat-free basis, closely followed by LF 3, and that the remaining birds had lower crude ash contents. HF 5, HF 8 and LF 3 had also the highest CaO contents, and at first sight these facts appear to be closely related to the calcium balance data as set out in Table IX. At the same time there is no reason to assume that the initial crude ash percentages of the birds were all identical, for the ash content of the mature Light Sussex pullet may vary from approximately 15·3 to 18·3 % of the dry fat-free carcass (Halnan, 1936). The final crude-ash compositions of the birds in the present experiment varied

*from 15·0 % in the case of HM 6 to 18·4 % in the case of* HF 5 when expressed as a percentage of the dry fat-free carcass, a variation of the same order as that recorded by Halnan. It is therefore impossible to assess the degree to which the final crude-ash percentages have been affected by the calcium balances, since even the final percentage figures are distributed over the recorded range of variation for normal mature Light Sussex pullets.

Before proceeding further with the consideration of the results of the calcium balance in relation to the carcass analyses, it may be pointed out that between 97·2 and 98·7 % of the total calcium in the bodies of the birds was present in the skeletons, in agreement with Halnan's (1937) statement. The data are given in Table VII.

Table VII

| | LF 2 | LF 3 | LM 1 | LM 4 | HF 5 | HF 8 | HM 6 | HM 7 |
|---|---|---|---|---|---|---|---|---|
| CaO in skeleton final g. | 29·062 | 33·686 | 28·545 | 22·108 | 38·128 | 39·285 | 21·726 | 24·088 |
| *CaO in fleshy parts, final g.* | *0·830* | *0·630* | *0·560* | *0·650* | *0·600* | *0·760* | *0·430* | *0·320* |
| $\frac{\text{CaO in skeleton}}{\text{Total body CaO}} \times 100$ | *97·2* | *98·5* | *98·1* | *97·2* | *98·5* | *98·1* | *98·1* | *98·7* |

It was not always easy to avoid removing minute spicules of bone along with the flesh during dissection. This source of error would have the effect of decreasing the calculated percentages, so that the figures 97·2 % for LF 2 and LM 4 are minimum values. The relevant data for HF 5 and HF 8, which had relatively hard bones, are probably the most accurate.

The data given in Table VII are particularly important in the case of HF 5 and HF 8, which stored considerable amounts of calcium before they were killed, because they demonstrate that no appreciable storage of calcium occurs elsewhere than in the skeleton. This was borne out by the analytical data for the various portions of the carcass. The data for the oviducts (Table VIII) are quoted as they confirm some previous observations (Buckner *et al.* 1924). It is clear that the oviduct has no storage functions in respect of calcium. The protein content, as might be expected, is variable, while the fat content is, on the whole, higher in group L.

The consideration of the data for the total calcium content of the carcasses in conjunction with the calcium balance data leads to results of considerable interest (Table IX).

Since the calcium retention (total calcium intake during the experi-

ment less total calcium in the excreta), the total calcium voided in eggs, and the final total amount of calcium in the carcass, together with the complete data for the daily calcium balance, were all known, it was possible to calculate (1) the initial gross amount of CaO in the bodies, and (2) the gross amount of CaO in the bodies at the onset of laying. It was then possible, by taking the difference between the final gross amount of CaO in the bodies and the gross amount at the onset of laying, to secure a figure for the "draft" on body calcium (i.e. on skeletal calcium, since about 98 % of the body calcium was in the skeletons) during the laying period.

Table VIII. *Analyses of hens' oviducts*

| | LF 2 | LF 3 | LM 1 | LM 4 | HF 5 | HF 8 | HM 6 | HM 7 |
|---|---|---|---|---|---|---|---|---|
| Moisture % | 76·12 | 78·86 | 75·66 | 73·20 | 73·20 | 77·61 | 75·04 | 78·24 |
| Protein % | 15·04 | 15·31 | 17·88 | 22·93 | 21·51 | 17·32 | 21·26 | 17·14 |
| Fat % | 7·54 | 4·19 | 5·32 | 3·64 | 3·90 | 3·58 | 2·66 | 3·73 |
| Ash % | 1·12 | 1·58 | 1·14 | 1·04 | 0·93 | 1·11 | 1·03 | 1·04 |
| CaO % | 0·029 | 0·025 | 0·031 | 0·028 | 0·035 | 0·035 | 0·046 | 0·034 |

Since the birds changed in weight during the experiment, the figures for "draft" in the case of those birds which were laying for a period (i.e. LM 1, LM 4, HM 6, and HM 7) are composite in the sense that "draft" on body CaO includes (1) CaO actually removed from the skeleton and (2) CaO deflected from purposes of new bone formation during the laying period to purposes of shell formation. At the same time the relative changes in weight during the laying periods were so small as to suggest that by far the greater part of the "draft" represents actual drafts on bone CaO.

The full relevant data are given in Table IX. It will be seen that all the birds, with the exception of LM 1, which laid on the fourth day of the experiment, stored calcium during the prelaying period. The amount of prelaying storage was, on the whole, greater in the case of group H; but was curiously low in the cases of HM 6 and HM 7. In the case of HM 7 this was, no doubt, partly due to the relatively short prelaying period.

Turning to the data for the calcium balances over the entire experimental periods, it will be seen that there was a heavy negative balance in the cases of LM 1 and LM 4 and a heavy positive balance in the cases of HF 5 and HF 8. In the case of LF 3 there was approximate calcium equilibrium, the calcium for the egg being offset by prelaying storage, whereas in the case of LF 2 prelaying storage was more than offset by the egg calcium owing to the fact that a second fully formed egg was

recovered from the oviduct at slaughter. Despite heavy laying, HM 6 showed a positive balance, while the negative balance was comparatively small in the case of HM 7.

When the data for "draft" on body calcium are considered, it will be seen that a greater or smaller "draft" on body calcium took place in every case with the exception of HF 8, which was actually able to continue storing calcium during the laying period. The "draft" was comparatively slight in the cases of HF 5 and HM 6.

Table IX. *Summary of balance data and calculation of "drafts" on body calcium during the laying periods*

| | LF 2 | LF 3 | LM 1 | LM 4 | HF 5 | HF 8 | HM 6 | HM 7 |
|---|---|---|---|---|---|---|---|---|
| Calcium retention for entire experimental period* g. CaO | 2·004 | 2·088 | 11·303 | 3·917 | 7·707 | 13·635 | 46·223 | 47·776 |
| *No. of eggs* | 2 | 1 | 13 | 11 | 1 | 1 | 18 | 19 |
| Calcium in eggs, g. CaO | 4·139 | 1·972 | 20·647 | 10·110 | 2·339 | 2·303 | 43·333 | 49·376 |
| Calcium balance for entire experimental period, g. CaO | –2·135 | 0·116 | –9·344 | –6·193 | 5·368 | 11·332 | 2·890 | –1·600 |
| Prelaying storage of calcium, g. CaO | 1·521 | 1·908 | 0·220 | 1·063 | 5·090 | 9·348 | 3·490 | 1·749 |
| Calcium in body, final, g. CaO | 29·892 | 34·316 | 29·105 | 22·750 | 38·738 | 40·045 | 22·156 | 24·408 |
| Calcium in body, initial, g. CaO | 32·027 | 34·200 | 38·449 | 28·943 | 33·370 | 28·713 | 19·266 | 26·008 |
| Calcium in body, at onset of laying, g. CaO | 33·548 | 36·108 | 38·669 | 30·006 | 39·460 | 38·061 | 22·756 | 27·757 |
| "Draft" on body calcium during laying period, g. CaO | 3·656 | 1·792 | 9·564 | 7·256 | 0·622 | –1·984† | 0·600 | 3·349 |
| "Draft" on body calcium *during laying period,* % | 13·0 | 5·0 | 24·7 | 24·2 | 0·2 | –5·2 | 0·3 | 12·1 |

* Retention signifies CaO in food less CaO in droppings.
† A negative "draft" is equivalent to storage.

Even when allowance is made for the fact that the data for "draft" are composite in the sense already mentioned, it is evident from the data for LM 1 and LM 4 that the pullet at the commencement of laying is capable of mobilizing almost a quarter of her total bodily calcium for purposes of egg formation, and this within a surprisingly short space of time.

The normal egg-laying cycle is markedly interfered with from the outset in birds on low calcium diets where food calcium must be supplemented with bodily calcium for egg formation, a fact which is always apparent in the records of times of laying. It is probable that, although the mobilization of skeletal calcium is rapid, it is not so rapid as the normal transference of calcium from ingested calcium carbonate to the eggshell; in addition the utilization of the skeletal source of calcium is complicated by the upset in acid-base metabolism occasioned by the necessity for simultaneous excretion of the bone phosphoric acid. In the cases of

LM 1 and LM 4, which were laying only soft-shelled eggs when killed, the bones were clearly no longer capable of supplying calcium rapidly enough. It is of interest to note that the percentage "draft" figures for these birds were 24·7 and 24·2 % respectively.

It is also evident from Table IX that prelaying storage under favourable conditions, as in the case of HF 8, can only enable provision to be made for some half-dozen eggs. The number might conceivably be increased by further inroads upon skeletal calcium, but the mobilization is doubtless slower in depleted birds.

Deobald *et al.* (1936) have estimated that about 10 % of the total body calcium of laying birds may be removed by depletion of skeletal reserves, an estimate considerably lower than the present one. From their data it can be seen that the tibia ash was reduced in laying birds from an average value (unweighted) of about 61·3 % to an average value (unweighted) of about 55·8 % by withholding calcium; this represents a reduction of about 9 % in the figure for percentage tibia ash. Unfortunately no data are given for the total calcium content of any of their birds, and it is not clear how they arrived at their estimate of a 10 % reduction in body calcium. It might appear that a reduction of 9 % in the figure for tibia ash percentage would indicate a reduction in total body calcium considerably greater than 10 %. In any case it is clear that, while reduction of the percentage tibia ash can serve as a gauge of depletion, any actual measurement of the amount of calcium lost from the body necessarily involves a knowledge of the total amounts of calcium present in the body before and after depletion.

In conclusion of this part of the discussion of experimental results it may be remarked that the data for the daily calcium and phosphorus balance confirmed previous observations (Common, 1936*a*) and need not be recapitulated in full. The retention ratios CaO : $P_2O_5$ are appended in Table X; the data are omitted in the case of LM 1, whose period of prelaying storage was very short.

Table X. *Retention ratio* CaO : $P_2O_5$ *during prelaying period*

| | LF 2 | LF 3 | LM 1 | LM 4 | HF 5 | HF 8 | HM 6 | HM 7 |
|---|---|---|---|---|---|---|---|---|
| Calcium retention g. CaO | 1·521 | 1·908 | — | 1·063 | 5·090 | 9·348 | 4·509 | 1·749 |
| Phosphorus retention g. $P_2O_5$ | 4·626 | 4·547 | — | 2·044 | 4·254 | 5·633 | 3·332 | 1·769 |
| Retention ratio $\frac{CaO}{P_2O_5}$ | 0·33 | 0·42 | — | 0·52 | 1·20 | 1·66 | 1·36 | 0·99 |

The retention ratios of group L are lower than those previously found with a ration similar to ration L (Common, 1936*a*). The retention

ratios of group H are, on the whole, lower than those previously found with a ration similar to ration H (Common, 1936*a*), but HF 8 displayed a very high value. The carcass analyses and bone analyses (see Table XI) preclude the possibility of the high retention ratio in this and other birds being due to storage of calcium carbonate; it is possible that such retention ratios may be due to phosphorus being withdrawn from other tissues for bone formation.

Table XI. *Inorganic constituents of bony tissues as percentage of dry fat-free skeleton*

| No. of bird | MgO total | $P_2O_5$ total | CaO total | CaO residual | $CO_2$ total | CaO as $CaCO_3$ | $\frac{CaO}{P_2O_5}$ |
|---|---|---|---|---|---|---|---|
| LF 2 | 0·577 | 22·81 | 30·34 | 4·13 | 2·85 | 3·63 | 1·330 |
| LF 3 | 0·555 | 23·60 | 31·90 | 4·72 | 3·17 | 4·03 | 1·352 |
| LM 1 | 0·555 | 21·66 | 28·43 | 3·54 | 2·75 | 3·50 | 1·313 |
| LM 4 | 0·538 | 22·68 | 29·99 | 3·87 | 2·72 | 3·46 | 1·322 |
| HF 5 | 0·552 | 23·64 | 32·64 | 5·41 | 3·27 | 4·16 | 1·381 |
| HF 8 | 0·535 | 23·29 | 32·11 | 5·27 | 3·31 | 4·21 | 1·378 |
| HM 6 | 0·474 | 22·25 | 30·13 | 4·44 | 2·75 | 3·50 | 1·354 |
| HM 7 | 0·511 | 22·03 | 29·85 | 4·47 | 2·95 | 3·75 | 1·355 |

| No. of bird | $\frac{\text{Carbonate CaO}}{\text{Total CaO}} \times 100$ | $\frac{\text{Residual CaO}}{\text{Total CaO}} \times 100$ | $\frac{\text{Residual CaO}}{\text{Carbonate CaO}}$ | $\frac{CaO}{MgO}$ | $MgO + CaO + P_2O_5 + CO_2$ |
|---|---|---|---|---|---|
| LF 2 | 11·96 | 1·36 | 1·14 | 52·6 | 56·58 |
| LF 3 | 12·63 | 1·48 | 1·17 | 57·5 | 59·23 |
| LM 1 | 12·31 | 1·25 | 1·01 | 51·2 | 53·40 |
| LM 4 | 11·54 | 1·29 | 1·12 | 55·7 | 55·93 |
| HF 5 | 12·75 | 1·66 | 1·30 | 59·2 | 60·10 |
| HF 8 | 13·11 | 1·64 | 1·25 | 60·0 | 59·25 |
| HM 6 | 11·62 | 1·47 | 1·27 | 63·5 | 55·60 |
| HM 7 | 12·56 | 1·50 | 1·19 | 59·5 | 55·34 |

Table XII. *Relative proportions of* CaO, MgO, $P_2O_5$, *and* $CO_2$ *in inorganic part of bone. Calculated as percentage of* CaO % + MgO % + $P_2O_5$ % + $CO_2$ %

| No. of bird | MgO | $P_2O_5$ | CaO | $CO_2$ | CaO residual | Gross balance g. CaO | "Draft" g. CaO |
|---|---|---|---|---|---|---|---|
| LF 2 | 1·020 | 40·31 | 53·62 | 5·04 | 7·30 | −2·14 | 3·66 |
| LF 3 | 0·927 | 39·84 | 53·86 | 5·35 | 7·92 | 0·12 | 1·79 |
| LM 1 | 1·039 | 40·56 | 53·24 | 5·15 | 6·63 | −9·43 | 9·56 |
| LM 4 | 0·962 | 40·55 | 53·62 | 4·86 | 6·92 | −6·18 | 7·26 |
| HF 5 | 0·918 | 39·33 | 54·31 | 5·44 | 9·00 | 6·82 | 0·62 |
| HF 8 | 0·903 | 39·31 | 54·19 | 5·59 | 8·89 | 13·09 | −1·98 |
| HM 6 | 0·852 | 39·98 | 54·19 | 4·94 | 7·99 | 5·76 | 0·60 |
| HM 7 | 0·923 | 39·81 | 53·94 | 5·33 | 8·08 | −0·72 | 3·35 |

## EXPERIMENTAL RESULTS, PART II

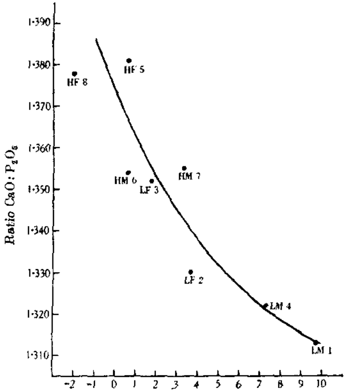
*The inorganic constituents of the skeletons*

The second object of the present experiment was to ascertain whether or not the storage and depletion of skeletal reserves of calcium for egg formation affected the relative proportions of calcium, phosphorus, magnesium and carbonate present in the skeleton. With this end in view the tibiae, femora, humeri, sterna and residual bony tissue from each bird were analysed separately; from these analyses the percentage compositions of the entire dry fat-free skeletons were calculated and set out in Table XI.

Although the composition of the inorganic part of bone is remarkably constant, it is known that the composition may be affected to some extent by species, age and diet. Morgulis (1931) has shown that the bones of fishes contain much less carbonate CaO than the bones of terrestrial vertebrates, and has associated this fact with the lower bicarbonate reserve in the blood of fishes. The work of other investigators (Fischler *et al.* 1931; Morgulis, 1931; Neal *et al.* 1931) has shown that the ratio $CaO : P_2O_5$, the ratio carbonate CaO : total CaO and the proportion of MgO all tend to increase with age. In the case of chickens, Harshaw *et al.* (1934) have traced an increase of the ratio $CaO : P_2O_5$ from 1·05 at hatching to 1·22 at 20 weeks. Alterations due to variations in the diet have also been reported. Magnesium restriction reduces the MgO content of the bones of rats (Orent *et al.* 1934), while Buckner *et al.* (1932) have shown that magnesium excess leads to an increased MgO content of the bone ash and to deformity of the skeleton. Buckner & Martin (1920) concluded that calcium deprivation did not significantly alter the percentage of CaO and $P_2O_5$ in the ash of the leg bones of laying hens, but examination of their data suggests that calcium shortage did in fact lower the ratio $CaO : P_2O_5$ in their experiments. Marek *et al.* (1934) have modified the composition of the inorganic part of the skeleton of swine by the use of rachitic acid and alkaline diets; their analyses show that the changes were not confined to the more recently deposited bone mineral, but also occurred throughout the bone. This latter observation is not surprising in view of the evidence that the mineral part of bone is part of a dynamic system (Chiewitz & Hevesy, 1935) and not merely a static deposit.

In addition to the percentage of CaO, $P_2O_5$, MgO and $CO_2$ in the dry fat-free skeletons certain ratios and derived figures computed from

the basal analytical data are given in Table XI. These derived figures were calculated by a conventional method which is essentially that followed by Morgulis (1931). Assuming that all the MgO was present as $Mg_3P_2O_8$, *the* $P_2O_5$ *present in this form was calculated and subtracted* from the total $P_2O_5$. The CaO equivalent to this residual $P_2O_5$ as $Ca_3P_2O_8$ was then calculated and subtracted from the total CaO, the remainder constituting the "residual CaO".



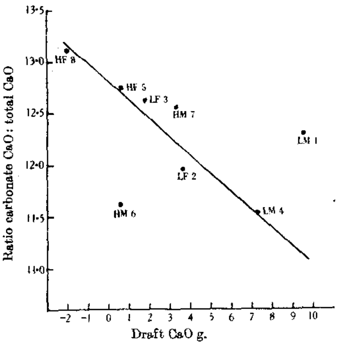

Graph 1.

When the compositions of the inorganic part of the bones are brought into relationship with the data for "draft" computed in Part I of the present paper, certain general relationships are apparent. These relationships are most easily expressed in the form of the accompanying graphs. The points on the graphs are annotated with the index letters and number *of the pullet concerned, so that effects due to diet may also be followed* on the graphs.

In Graph 1 the ratio CaO : $P_2O_5$ has been plotted against "draft" on body CaO. There is a close relationship, and it may be inferred that heavy calcinm depletion in the fowl during egg production lowers the *ratio* CaO : $P_2O_5$, *while storage of* CaO *on a ration high in* $CaCO_3$ *raises*

the ratio. This is not likely to be due to differences in organically bound $P_2O_5$, for Burns & Henderson (1935) have shown that fat-free bone contains practically no organic phosphorus.

To a certain extent this relationship is due to differences in the calculated $CaCO_3$ content of the bones, as may be seen when the ratio carbonate CaO : total CaO is plotted against "draft" as in Graph 2. It will be noted, however, that LM 1 and HM 6 depart from the general tendency shown by the other birds. This is possibly due to the long period of slow calcium storage enjoyed by LM 1 between its two laying periods,



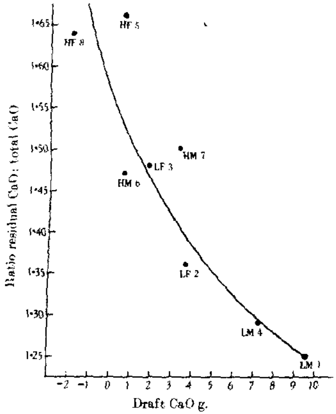

Graph 2.

but the reason for the deviation is less clear in the case of HM 6. The latter bird was also noteworthy for the low content of MgO in its skeleton.

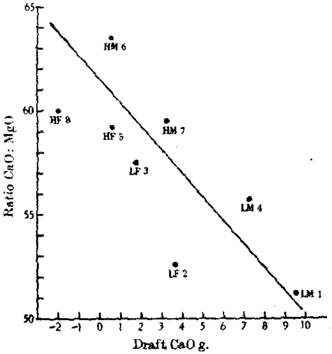
The ratios carbonate CaO : total CaO in the skeleton are considerably higher than those quoted by Halnan (1937) for the bones of poultry; the reason for this discrepancy is not clear, but the present data agree in general with those usually found in other terrestrial vertebrates.

A very much better relationship is seen when the ratio "residual CaO" : total CaO is plotted against draft, as in Graph 3, and in this case LM 1 and HM 6 fall into line.

When the ratio CaO : MgO is plotted against draft a further relationship is apparent (Graph 4). Heavy "draft" is associated with a lowered ratio. Two possible interpretations may be placed on this fact: either




Graph 3.




Graph 4.

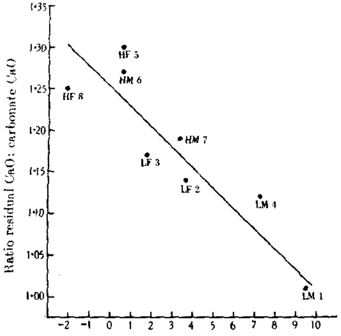
(1) magnesium tends to replace calcium in bones subjected to heavy depletion or (2) magnesium is relatively difficultly deposited and relatively difficultly removed from the bones as compared with calcium. It seems clear from the foregoing graphs that the compositions of the mineral constituents of the skeleton were in fact modified by depletion of the skeletal reserves of calcium. The modifications took place relatively quickly, for in no case were the experimental periods very long.

The interpretation of these modifications cannot be made so easily, and is partly bound up with the vexed question of the composition of the bone salt (Shohl, 1933; Robison, 1937). It is not easy to distinguish the possible effects of differences in acid-base metabolism from the effects of depletion, yet on the whole it appears that depletion tended (*a*) to lower the ratio $CaO : P_2O_5$, (*b*) to lower the ratio residual CaO : total CaO, (*c*) to lower the ratio carbonate CaO : total CaO, (*d*) to decrease the ratio CaO : MgO, and these effects were evident in both group L and group H. Similar effects were produced by ration L as compared with ration H, i.e. by a more acidotic diet.

The variations in the composition of the inorganic part of the skeleton are not easily seen from the percentage figures for CaO, $P_2O_5$, MgO and $CO_2$ given in Table XI. A clearer picture is obtained when these data are reduced to a common basis by reckoning them as percentages of the sum of the CaO, $P_2O_5$, MgO and $CO_2$ present. The data of Table XI as recalculated in this way are set out in Table XII.

It will be noticed from Table XI that the "residual CaO" is always slightly in excess of the carbonate CaO as calculated from the $CO_2$ content of the skeleton. This latter calculation has been criticized by Klement (1934), but it has been shown (Marek *et al.* 1935) that the carbonate of bone is almost all present as carbonate of alkaline earths, alkaline carbonates being unable to account for more than very small amounts, and that chiefly in the bones of very young animals, which are poor in carbonate and rich in alkalis. Marek and his co-workers (1934) made substantially similar calculations and claimed to have found good agreement between residual CaO and CaO equivalent to the $CO_2$ present, but it may be remarked that their data show a distinct tendency for the "residual CaO" to be slightly higher except in the case of swine receiving an acid diet, the difference being greatest with the swine receiving a high calcium diet. Fischler *et al.* (1931) observed a somewhat similar excess of residual CaO, and ascribed it to the presence of small amounts of calcium soaps as normal constituents of bone. Morgulis (1931) observed a similar and much larger excess, but the analyses were

made on bone ashed by the alkaline glycerol method, a procedure which has been severely criticized. However, when the ratio residual CaO : carbonate CaO is calculated for the data of the present experiment, an unexpected relationship between the ratio and the draft figures was obtained, as may be seen from Graph 5. Apparently the "residual CaO" is depleted more rapidly than carbonate CaO, and the two coincide only in the case of LM 1, which was at once heavily depleted and also probably underwent more severe and prolonged acidosis than the other birds owing to its comparatively long experimental period.




Graph 5.

In conclusion it can be said that the present experiment strongly suggests that the composition of the inorganic part of the bones of poultry may be modified within a comparatively short space of time by differences in mineral metabolism associated with the calcium metabolism of egg production and differences in calcium carbonate intake in the food.

It is possible that the modifications of bone composition are related to the various changes which take place in the calcium and phosphorus compounds of the blood (Halnan, 1937; Laskowski, 1935) and of serum phosphatase (Auchinachie & Emslie, 1934; Common, 1936*b*) during laying, and to the modifications of these changes which can be induced by altering the calcium carbonate intake in the food. Such a view receives support from the work of Logan & Taylor (1937), who have adduced

evidence in favour of the view that the bone salt is formed step by step, with adsorption phenomena modifying the composition of the salts deposited. At the same time it must be re-emphasized that chemical analyses alone cannot solve the nature of the bone salts; chemical analyses can, however, detect changes in the proportions of the elements present, and any theory of the bone salt and its deposition must necessarily explain such differences as those noted in the present experiment.

## Summary

1. An experiment on pullets combining daily calcium and phosphorus balance determinations with carcass analyses is described.

2. It is shown that fat content of pullets is affected by the calcium content of the ration. The fat content was higher on a low calcium ration than on a high calcium ration.

3. The CaO content of pullets was raised considerably by feeding a high calcium ration before laying. This CaO must have been stored in the bones, which contained between 97·2 and 98·7 % of all the CaO in the body.

4. The experiment suggests that pullets may use up to about one-quarter of their body calcium at the outset of laying for purposes of shell formation.

5. The composition of the inorganic material of the skeleton of pullets may be modified by alterations in their mineral metabolism due to egg production on diets with and without a calcium carbonate supplement. The nature of these modifications and their relationship to calcium metabolism during laying is discussed.

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# COMPRESSIBILITY CURVES AS A QUANTITATIVE MEASURE OF SOIL TILTH, II

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(With Four Text-figures)

## Introduction

In an earlier paper (Scott Blair, 1937)[1] methods were described for obtaining load-deformation ($S/\sigma$) curves for soils compressed by lowering gradually on to the soil surface a cylindrical metal weight hung from its centre of gravity. Apparatus were described for obtaining these curves both in the field and in the laboratory, and in the latter case also for plotting automatically a curve[2] for $\sqrt{S}/\sigma$.

Since the form of these curves was shown to be closely connected with the tilth of the soil (Scott Blair, 1937), it appeared desirable to carry out experiments to study the effects of cultivation, and of time of resting since cultivating on the compression curves of soils *in situ*. For this purpose an allotment which had been in cultivation for many years was selected. A rectangle of sides 42 ft. × 28 ft. was carefully dug with a fork to a depth of about 8 in., and divided up into twenty-four squares of length 7 ft. numbered as shown by the arabic figures and letters in Fig. 1.

During the eight weeks following the digging, six convenient days were selected (nos. I–VI) and on each of these days a compression experiment was carried out on four of the twenty-four plots, one in each of the rows *a*–*d*, except on the fourth and fifth days when two plots were

[1] Curves of very similar form likewise related to soil tilth have recently been obtained by Hénin (1937) using a penetrometer.

[2] Since the publication of Part I (Scott Blair, 1937), one of us (G. H. C.) has pointed out that the function of load plotted against deformation in the laboratory technique is not a simple square root, due to the presence of a flat surface at the bottom of the mercury bucket. This correction gives more negative values of the parameter $\gamma$ throughout, and although the general conclusions remain unaltered, in any further use of the apparatus arrangements will have to be made to ensure that the simple square-root function is plotted.

taken in the same row. In Fig. 1 each plot is marked with a Roman figure corresponding to the day on which the test was carried out.

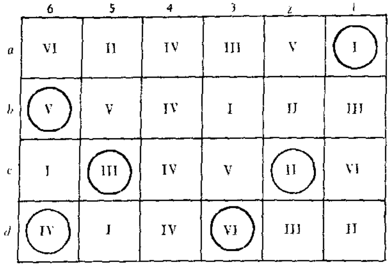


Fig. 1.

THE NATURE OF THE TESTS

The compression tests were carried out as described in the earlier paper except that it was decided for this preliminary investigation to consider only plastic deformations. The total load of 230 lb. was therefore applied in increments of about 12 lb. at intervals of 15 sec. After the full load had been given to the soil, an interval of 1 min. was allowed to elapse, and a measure of further deformation during this time was obtained. In the case of three of the four plots tested on each of the six chosen days, the soil was thoroughly redug with a fork after the loading, and the test immediately repeated. Although the whole area had been dug at the start of the experiment, the soil in its condition before digging on the day of the test will, for convenience, be referred to as "undug soil". One plot in each of the six rows (1–6) was chosen at random, and in these plots were buried at the time of the initial digging lengths of about 20 ft. each of rubber tubing, of diameter about 5 mm., at about half the total depth of the digging. The tubing was laid in such a way as to be fairly evenly distributed over the part of the plot on which the loading tests were done, and the two ends were led out together to a point on the edge of the plot. The plots so treated are marked with rings in Fig. 1, and will be referred to as "rubbered plots". In order not to disturb the rubber tubing, these plots were not dug after loading, so that only three replicates on newly dug soil were obtained on each day's test, as compared with four for undug soil.

The extent to which the rubber tubes were compressed by the surrounding soil was measured from day to day during the two months following the initial digging by observing the time of flow of 100 c.c. of water at constant temperature (20° C.)[1] falling through a given head (12·5 cm.). The rubber tubes were kept full of water, and were flushed out with water at a suitable temperature before testing until the required temperature was obtained. The results of this test were recorded in seconds, and are means of two or three replicates.

Care was taken during the course of the experiment to walk only along the extreme edges of the plots in the experiment except in the case of the plot being tested by loading. This plot was in every case thoroughly trampled during the loading experiment, and in the case of the rubbered plots the effects of trampling and subsequent recovery, if any, could be observed. All the plots still under experiment were kept as clean as possible by careful hand-weeding. Soil temperatures were recorded each day when readings were taken (except, unfortunately, right at the start of the experiment), and moistures were taken from each plot at the time of the compression test. The area was subject to natural weathering, except that at one period when the soil was very dry, an artificial irrigation of 10 gal. of water was applied by hose to each plot in the experiment in as standard a manner as possible. Samples were taken from time to time from the edges of the plots to the laboratory and were very carefully transferred to trays for testing by the laboratory method (Scott Blair, 1937). For soils in a fine degree of comminution, the laboratory technique gives an encouraging indication of tilth (Scott Blair, 1937*a*), but in the case of the present experiment the soil lumps tended to he large compared with the size of the loading cylinder, and the curves were highly irregular especially when the soils were rather dry, as they tended to be during the later part of the experiment.

By the means described above it was hoped to study:

(1) The effects of time since cultivation on the various factors associated with soil compressibility.

(2) The extent to which these effects can be removed by a further simple digging.

(3) The immediate and long-time effects of compressing soil on its degree of compression.

(4) The extent to which all these factors depend on soil moisture and, in some cases, on temperature.

[1] Unfortunately adequate temperature control was lacking during the first few days the experiment.

It is fully appreciated that an experiment on a single soil over the short period of two months under the simplest cultivation processes can give at best only a preliminary indication either of the changes in tilth produced by cultivation and resting in general, or of the potentialities of the compression method to measure such changes. Interesting indications of the processes taking place are, however, already forthcoming.

### METHOD OF ANALYSING COMPRESSION CURVE DATA

A typical compression curve is shown in Fig. 2. The complexity of the curve precludes any exact mathematical analysis, and an empirical method for deriving certain characteristics of the curve is used.

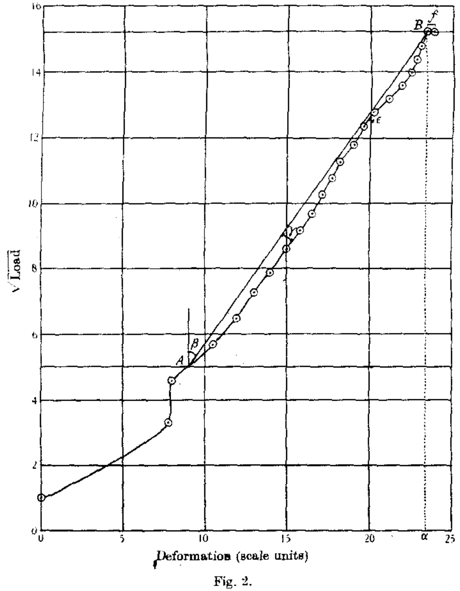


Fig. 2.

The first factor is clearly the total deformation during the loading ($\alpha$). This is expressed as the number of scale units traversed by the indicator on the deformation scale during the whole process of loading (Scott Blair, 1937). Each scale unit $\equiv$ 0·198 cm. of soil compression. The total deformation recorded depends on the correct fixing of the zero, i.e. the point at which the weight first comes into contact with the soil. This is not only difficult to determine, but is largely dependent on the size of the largest lumps on the soil surface. A general measure of compressibility is better expressed by the slope of the main part of the curve. For this purpose, a line is drawn joining the points $A$ and $B$ in Fig. 2, where $A$ is the point where the curve crosses the ordinate corresponding to a load of 25 lb. ($\sqrt{S}=5$), and $B$ corresponds to the final load and deformation. The slope of the line $AB$ with respect to the $\sqrt{S}$ axis ($\beta$) gives the best general measure of true compressibility.

In the earlier papers (Scott Blair, 1937, 1937$a$) the curvature of the compression curve was discussed. This can be roughly expressed as the maximum linear distance ($\gamma$) between the experimental curve and the line $AB$, taken as positive when the curve falls below the straight line, and negative when above. For curves which are sigmoid in type and fall roughly symmetrically about $AB$, $\gamma$ is taken as zero. The experimental curve often shows a wavy form due to discreet increments in deformation occasioned by the crushing or displacement of individual lumps. The maximum amplitude of the wave-form is given as $\epsilon$. Finally, the flow during 1 min. rest under full load, expressed in deformation scale units, is designated $f$. The parameters $\alpha$, $\beta$, $\gamma$, $\epsilon$ and $f$ give a fair indication of the principal characteristics of the loading curve.

It has been shown (Scott Blair, 1937, 1937$a$) that in the laboratory test, good soil tilth is associated with a high compressibility (high $\alpha$ and $\beta$) together with $\gamma$'s tending towards positive values. A wet structureless soil may be highly compressible, but shows a negative $\gamma$, whereas a dry powdery soil or a soil consisting of hard incompressible lumps gives low $\alpha$ and $\beta$ values as well as negative $\gamma$. There is an optimum value for $\epsilon$: too small a value indicating small or very soft crumbs, and too large a value showing the presence of large intractable lumps.

### Results of the experiment

Values of $\alpha$, $\beta$, $\gamma$, $\epsilon$, $f$ and moisture ($M$, expressed on dry basis) for all the plots are given in Table I.

Table I

| Date | Plot | $\alpha$ | $\beta$ | $\gamma$ | $\epsilon$ | $f$ | $M$ |
|---|---|---|---|---|---|---|---|
| 2. vi. 37 | 1a *u | 17·8 | 36 | +1·05 | 0·60 | 0·40 | 22·6 |
| | 3b u | 23·4 | 36 | +0·40 | 0·35 | 0·45 | 22·8 |
| | 3b d | 13·9 | 28 | +0·30 | 0·20 | 0·35 | — |
| | 5d u | 16·0 | 35 | −0·30 (*S*) | 0·50 | — | 23·1 |
| | 5d d | 21·0 | 38 | +0·40 | 0·35 | 0·55 | — |
| | 6c u | 16·6 | 27 | −0·35 | 0·35 | 0·70 | 22·5 |
| | 6c d | 18·9 | 38 | −0·35 | 0·25 | 0·45 | — |
| 9. vi. 37 | 1d u | 14·0 | 32 | +1·60 | 1·10 | 0·25 | 16·3 |
| | 1d d | 14·7 | 31 | −0·60 | 0·20 | 0·45 | — |
| | 2b u | 11·5 | 28 | −1·05 | 0·50 | 0·45 | 17·6 |
| | 2b d | 20·9 | 37 | +0·20 | 0·10 | 0·70 | — |
| | 2c *u | 16·0 | 25 | −0·60 (*S*) | 0·50 | 0·20 | 17·2 |
| | 5a u | 15·2 | 35 | +1·15 | 1·10 | 0·70 | 17·2 |
| | 5a d | 19·4 | 35 | +0·55 | 0·30 | 0·55 | — |
| 21. vi. 37 | 1b u | 17·7 | 27 | +0·40 | 0·20 | 0·40 | 20·0 |
| | 1b d | 20·6 | 36 | 0·00 (*S*) | 0·20 | 0·60 | — |
| | 2d u | 22·3 | 43 | +0·95 (*S*) | 0·60 | 0·90 | 20·3 |
| | 2d d | 15·1 | 29 | 0·00 (*S*) | 0·05 | 0·55 | — |
| | 3a u | 14·4 | 31 | +0·75 | 0·45 | 0·45 | 18·1 |
| | 3a d | 23·9 | 42 | +0·50 | 0·20 | 0·65 | — |
| | 5c *u | 14·9 | 29 | −0·75 | 0·35 | 0·55 | 20·5 |
| 2. vii. 37 | 4a u | 6·5 | 16 | −0·42 | 0·10 | 0·45 | 5·6 |
| | 4a d | 16·9 | 34 | +0·30 | 0·20 | 0·45 | — |
| | 4c u | 16·2 | 22 | +0·30 | 0·25 | 0·20 | 4·9 |
| | 4c d | 23·3 | 33 | +0·58 | 0·15 | 0·40 | — |
| | 4d u | 11·7 | 15 | +0·55 (*S*) | 0·70 | 0·15 | 5·1 |
| | 4d d | 18·9 | 36 | +0·70 | 0·40 | 0·40 | — |
| | 6d *u | 11·9 | 25 | 0·00 (*S*) | 0·75 | 0·15 | 4·7 |
| 15. vii. 37 | 2a u | 17·0 | 30 | 0·00 (*S*) | 0·70 | 0·20 | 13·2 |
| | 2a d | 13·6 | 27 | +0·30 | 0·15 | 0·40 | — |
| | 3c u | 16·3 | 27 | +0·60 | 0·30 | 0·35 | 13·3 |
| | 3c d | 16·2 | 31 | +0·20 | 0·05 | 0·50 | — |
| | 5b u | 13·1 | 28 | +0·20 | 0·30 | 0·40 | 16·2 |
| | 5b d | 17·5 | 33 | 0·00 (*S*) | 0·10 | 0·40 | — |
| | 6b *u | 13·7 | 22 | +0·20 | 0·10 | 0·35 | 18·7 |
| 24. vii. 37 | 1c u | 10·3 | 24 | +0·10 | 0·10 | 0·50 | 19·3 |
| | 1c d | 17·0 | 34 | +0·25 | 0·20 | 0·65 | — |
| | 3d *u | 14·4 | 29 | 0·00 (*S*) | 0·40 | 0·45 | 22·9 |
| | 4b u | 11·2 | 26 | 0·00 (*S*) | 0·20 | 0·45 | 22·3 |
| | 4b d | 18·8 | 34 | 0·00 (*S*) | 0·20 | 0·60 | — |
| | 6a u | 10·2 | 23 | −0·80 | 0·60 | 0·45 | 19·3 |
| | 6a d | 14·6 | 33 | −0·20 | 0·15 | 0·60 | — |

* Rubbered plots. *u* = undug; *d* = dug.

The mean values for each day are plotted in Fig. 3.

The results of the rubber-tubing experiment are given in Fig. 4, in which time of flow is plotted against date. Arrows indicate the date

at which compression tests were carried out. The rainfall figures are given in Table II.

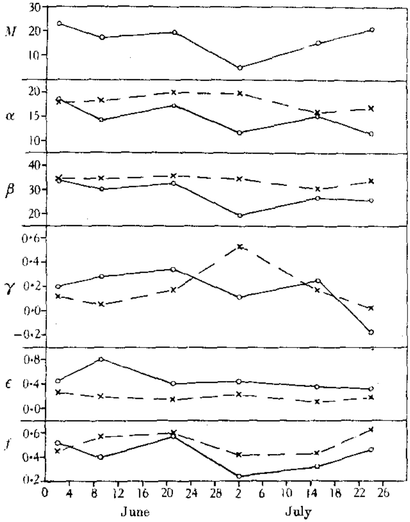


Fig. 3. o——o undug. ×— —× dug.

Table II

(On dates not quoted no rain fell)

| | June | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Date | 8 | 10 | 12 | 13 | 15 | 16 | 18 | 19 |
| Rainfall (in.) | 0·06 | 0·38 | 0·66 | 0·25 | 0·01 | 0·01 | 0·02 | 0·17 |

| | June | | | July | | | | |
|---|---|---|---|---|---|---|---|---|
| Date | 20 | 22 | 29 | 5 | 6 | 9 | 10 | 11 |
| Rainfall (in.) | 0·01 | 0·23 | 0·04 | 0·04 | 0·20 | 0·19 | 0·05 | 0·02 |

| | July | | | | | | Aug. |
|---|---|---|---|---|---|---|---|
| Date | 14 | 15 | 19 | 21 | 22 | 23 | 12 |
| Rainfall (in.) | (0·40)* | 0·59 | 0·31 | 0·07 | 0·08 | 0·24 | 0·09 |

* Irrigation.

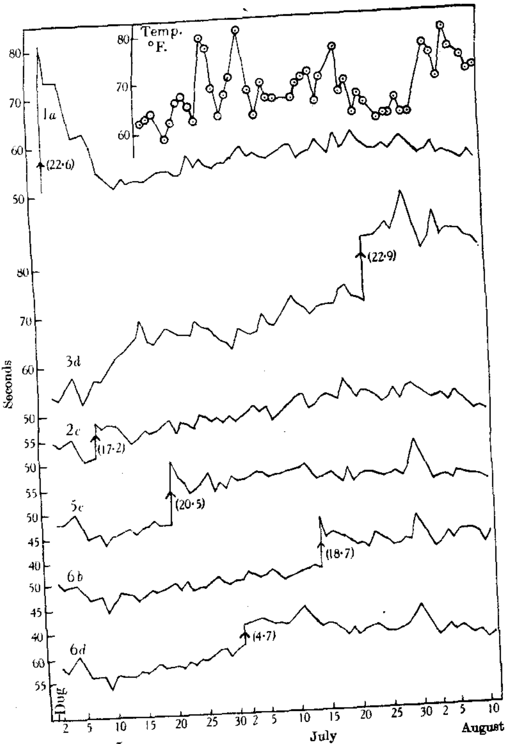


Fig. 4. Time of flow of 100 cc. water through rubber tubes for all six plots.

## DISCUSSION OF RESULTS

As previously stated, $\beta$ gives the best general measure of compressibility, but it is surprising, in view of the difficulties in fixing a zero, that mean figures for $\alpha$ follow those for $\beta$ fairly closely. In the case of undug soils, $\alpha$ falls during the experiment. This is due partly to the fact that moisture content was tending to fall during the earlier period, but it is probable that the time since cultivation also had its effect. The $\beta$ curve has a very similar form, and is doubtless affected by moisture and time in a similar way. $\gamma$ also falls during the experiment, and has a positive value during almost the whole of the period. Although it may not be unrelated to moisture, it is probable that the fall in $\gamma$ reflects a gradual deterioration in tilth with the lapse of time after cultivation. Since the initial tilth of the area was good, this is in agreement with the earlier finding that good tilth is associated, amongst other things, with positive $\gamma$ values. It will be seen from Fig. 2 that $\epsilon$, which gives a measure of lumpiness, can only be assessed roughly. It appears to fall slightly during the experiment. $f$ (residual flow) follows the moisture content very closely.

In the soils dug immediately after loading, and recompressed, $\alpha$ and $\beta$ show no marked dependence on moisture content or time. The values are in both cases higher than those for the undug soil except right at the start of the experiment. This suggests that the short periods during which the soil has rested since cultivation do not affect its tilth in any way that cannot be reversed by a single digging. The fact that $\alpha$ and $\beta$ are hardly affected by moisture content in newly dug soils suggests that their compression consists mainly in a repacking of crumbs without much plastic deformation or rupture.

The $\gamma$ curve is much less regular. Local soil variations which, as seen in Table I, show marked effects on all the parameters, cause their widest divergence in the case of $\gamma$, and it is doubtful whether the apparent inverse relationship observable in Fig. 3 between $\gamma$ for dug and undug soils has any real meaning.

$\epsilon$ is much reduced by digging, and for dug soils is almost independent of moisture and time.

With the exception of the first point which is abnormally low, $f$ values for dug soils are higher than undug values, and, unlike all the other parameters, they follow moisture contents fairly closely. This is doubtless due to the fact that $f$ is, more than any other parameter, mainly independent of the surface properties of the soil.

The method of measuring soil compression by taking the time of flow of a fixed quantity of water through a rubber tube buried in the soil gives data which, for the actual dates when compressions were carried out, do not appear to be correlated with any of the parameters discussed above. The rubber-tube method gives a static measure of compression, whereas the loading method measures compressibility dynamically, but even allowing for this, it is surprising that a closer connexion between the results of the two methods is not apparent. There are certain trends in the rubber tube experimental curves, however, which do correspond to changes in the compression parameters, but this correspondence is very limited. The rubber-tube method suffers from the disadvantages that a compression by a single stone or root at one point on the tube will produce an effect out of all proportion to its importance, and that it is difficult to ensure that the tests are carried out at a temperature sufficiently near to that postulated. The following general trends in the curves are noticeable. During the first few days of the experiment, most of the values show a tendency to fall. Unfortunately temperature control before 10 June was not adequate to allow of any conclusions as to the significance of this. A separate test started later on a neighbouring piece of soil with careful temperature control did not show any such fall. Following this apparent fall there is a progressive rise in all cases up to the time when the soil is loaded and trampled. This is more marked in some plots than in others, and corresponds to the increase in degree of compression also measured by the changes in $\alpha$ and $\beta$. In Fig. 4, moisture contents at the time of compression are given in brackets. It will be seen that, although no special precautions were taken to ensure that the same amount of trampling on the plots took place in all cases, the changes in compression recorded by the rubber-tube tests depended largely on moisture content. Immediately following loading, there are indications in some of the curves, especially in 1*a*, of an apparent immediate decrease in compression, indicating some sort of slow elastic recovery, although it must be remembered that the accuracy of the first few points following the loading of 1*a* is unsatisfactory. Following this fall, the curves tend to rise showing increased compression, but, rather surprisingly, they pass through maxima, and in the later part of the experiment they fall. The maxima appear to occur at about the same date, independent of the treatment of the soil. It will be seen that there is no correlation with soil temperature, as given in Fig. 4, or with rainfall (Table II). It is possible that the apparent loosening may be due to the tendency of the rubber tubes to

rise in the soil, and in certain places even to appear on the surface, though there is no evidence that the effect was more marked in the plots where this occurred, notably 6*d* and 5*c*. In addition, some loosening of the soil may have occurred, due to the continued removal by hand of small weeds as they appeared.

## CONCLUSIONS

Experiments conducted on an allotment soil are in general agreement with conclusions derived largely from laboratory experiments in the earlier papers (Scott Blair, 1937, 1937*a*) about the relationship between compressibility curves and soil tilth. Good tilth is associated in general with high total compressibility, a curve concave to the deformation axis plotting $\sqrt{S}/\sigma$, and a waviness of a certain amplitude indicating lumps of reasonable size. After soil is dug, it gradually settles into a more compact condition. This process takes many months, and the experiment described only followed the first part, nor were any changes in tilth produced which could not be reversed by a single further digging. The behaviour of the soil after compression and trampling, as indicated by the rubber-tube technique, is not yet fully understood, but there is no doubt that still further compacting takes place. The compression parameters were closely correlated with soil moisture, with the interesting exception that newly dug soil showed $\beta$ values more or less independent of moisture.

We conclude that compression methods provide an effective way of studying changes in soil conditions in the field. The loading technique appears to be satisfactory, but the rubber-tube technique still leaves much to be desired, and it is hoped to bring about further improvements. Experiments should be carried out over longer periods, providing a greater mass of data in order that a more complete analysis may be effected.

## SUMMARY

1. The method described in an earlier paper for measuring the compressibility of soils *in situ* has been used to study the gradual consolidation of soil following digging with a fork, and a new method in which the rate of flow of water through rubber tubes buried in the soil gives a measure of compression, is described.

2. By means of this latter method some measure can be obtained of the changes that take place in the soil after it has been loaded and trampled.

3. The results of the experiments confirm and amplify the earlier conclusions. Without further data it is hard to distinguish quantitatively the effects of moisture and time, but it is of interest that whereas the compressibility of newly dug soils is hardly affected by differences in moisture for the range of stress used, that of soils which have rested for some time since cultivation is much increased by an increase in moisture content.

4. Experiments on a wider scale should be undertaken in order that a more complete analysis may be effected.

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# SOME OBSERVATIONS ON THE NORMAL VARIATIONS IN COMPOSITION OF LIGHT SUSSEX COCKERELS

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(With Six Text-figures)

## Introduction

In carrying out feeding experiments designed to test the relative efficiencies of feeding stuffs for conversion into energy and protein it is recognized that certain difficulties exist which may render impossible interpretation of the results obtained.

The difficulties are twofold. First, live-weight increase measurements are unreliable owing to the variability in the composition of the live-weight increases obtained. Secondly, the variation in the composition of the original material used may be such as to render untrustworthy results obtained by the slaughter method. In this latter method, a number of animals are slaughtered at the beginning of the experiment and the average composition of the slaughtered individuals is taken as representative of the composition of the survivors used in the trial. In determining the number of individuals necessary to give a true value of the average composition of the survivors, it is obviously necessary to know to what extent variation in individual carcass composition occurs, and whether the variation in composition is so large as to render this method of experimentation impracticable, as suggested by Gutteridge (1937). It was with the view of studying the extent and nature of the variation in carcass composition of fowls that the investigation detailed below was undertaken.

## Experimental material

The fowls used in this investigation were the forty surviving cockerels of a Light Sussex flock which had been subjected to the same sy tem of feeding and management throughout. The birds had been hatched in

an incubator, reared in brooders, and placed out on range in Sussex arks. At the time of killing, thirty-four were 158 days old and six 154 days old. The birds were killed by dislocation of the neck, plucked, packed in wooden boxes, and stored in a temperature of $-10^\circ$ C. until withdrawn for analysis.

## Method of analysis

Each bird was weighed immediately after leaving cold store, allowed to thaw overnight, reweighed after thawing, and then dissected. After preliminary dissection and weighing of the pectoral muscles, the digestive tract was removed and emptied of its contents. The body was then dissected into bones, flesh, and offal, the bones consisting of the skeleton with as much as possible of the adherent flesh removed, the flesh consisting of the meat and skin, and the offal consisting of the intestines, gizzard, heart, liver, spleen, stomach, trachea, lungs, testes, oesophagus and crop. Slight losses on dissection were considered as flesh.

### *Sampling of flesh and offal for analysis*

The entire sample in each case was thoroughly minced and mixed, and 1000g. weighed out into a flat dish, soaked in alcohol, and a few drops of toluol added. After drying in a steam oven the dried sample was reweighed, ground into a fine powder, and bottled for analysis.

### *Sampling of the bones*

The entire sample of bones was dried in a steam oven, being broken during the process, and then reweighed. The entire sample was then passed through a bone crusher and thoroughly mixed. 100 g. of the mixed dried bones were then extracted in the cold with ether, the extract and washings being made up to 500 c.c. The fat in an aliquot portion of the ether extract was then determined. The dried extracted residue was then finely ground and bottled for analysis.

### *Methods of analysis*

For the determination of the protein, fat, moisture and ash the usual analytical methods were used, ashing being carried out at a dull red heat.

For the determination of the energy values of the carcass, the factors 9·43 Cal. per g. of fat and 5·34 Cal. per g. of protein were used. The value for chicken fat was obtained by means of bomb calorimeter determina-

tions on samples of chicken fat, and the value for protein was extracted by difference from forty bomb calorimeter determinations on samples of flesh and offal of which the protein and fat contents were known. The value for protein per gram was 5·34 ± 0·09 Cal.

Table I. *Experimental data.* (*Weights in grams*)

| No. of bird | Live wt. | Plucked weight | Empty plucked wt. | Wt. of flesh | Wt. of offal | Wt. of bones | Wt. of breast muscle | Cal. per 100 g. live wt. |
|---|---|---|---|---|---|---|---|---|
| 305 | 2406 | 2204 | 2143 | 1237 | 238 | 668 | 251 | 144 |
| 310 | 2063 | 1918 | 1852 | 1035 | 254 | 563 | 214 | 142 |
| 312 | 2304 | 2110 | 2051 | 1130 | 224 | 697 | 229 | 153 |
| 313 | 1845 | 1705 | 1644 | 901 | 198 | 545 | 166 | 115 |
| 314 | 1977 | 1818 | 1757 | 985 | 202 | 570 | 192 | 139 |
| 315 | 2640 | 2435 | 2373 | 1372 | 244 | 757 | 280 | 156 |
| 321 | 2576 | 2351 | 2289 | 1341 | 270 | 678 | 277 | 166 |
| 322 | 2256 | 2055 | 2001 | 1210 | 216 | 575 | 257 | 157 |
| 323 | 2438 | 2240 | 2170 | 1256 | 226 | 688 | 274 | 158 |
| 326 | 2034 | 1848 | 1804 | 1042 | 205 | 557 | 194 | 174 |
| 328 | 1453 | 1355 | 1300 | 671 | 156 | 473 | 95 | 117 |
| 329 | 2480 | 2273 | 2219 | 1336 | 250 | 633 | 245 | 148 |
| 331 | 2412 | 2231 | 2174 | 1268 | 225 | 681 | 260 | 151 |
| 332 | 2172 | 1993 | 1898 | 1097 | 230 | 571 | 236 | 143 |
| 337 | 1973 | 1790 | 1729 | 941 | 206 | 582 | 179 | 138 |
| 342 | 1674 | 1519 | 1455 | 777 | 200 | 478 | 143 | 140 |
| 343 | 1855 | 1682 | 1623 | 905 | 198 | 520 | 168 | 132 |
| 347 | 2366 | 2156 | 2088 | 1232 | 247 | 609 | 254 | 187 |
| 348 | 2596 | 2370 | 2319 | 1361 | 245 | 713 | 269 | 189 |
| 350 | 2666 | 2443 | 2392 | 1287 | 304 | 801 | 193 | 177 |
| 354 | 2047 | 1895 | 1855 | 1055 | 193 | 607 | 161 | 156 |
| 355 | 2392 | 2245 | 2174 | 1245 | 208 | 721 | 238 | 144 |
| 358 | 2158 | 1960 | 1911 | 1085 | 230 | 596 | 208 | 148 |
| 359 | 2483 | 2308 | 2242 | 1277 | 236 | 729 | 240 | 162 |
| 364 | 2080 | 1887 | 1844 | 1074 | 224 | 546 | 201 | 184 |
| 369 | 2086 | 1926 | 1879 | 1076 | 204 | 599 | 221 | 149 |
| 370 | 2224 | 2057 | 2002 | 1186 | 197 | 619 | 251 | 144 |
| 375 | 1996 | 1847 | 1800 | 995 | 227 | 578 | 178 | 128 |
| 376 | 2462 | 2253 | 2175 | 1219 | 242 | 714 | 243 | 149 |
| 385 | 1964 | 1859 | 1811 | 1083 | 171 | 557 | 184 | 136 |
| 386 | 1912 | 1728 | 1703 | 982 | 181 | 536 | 185 | 161 |
| 391 | 1806 | 1693 | 1644 | 907 | 209 | 528 | 180 | 161 |
| 393 | 2445 | 2256 | 2216 | 1295 | 218 | 703 | 260 | 148 |
| 394 | 2138 | 1971 | 1901 | 1059 | 228 | 614 | 197 | 160 |
| 404 | 2093 | 1864 | 1836 | 1067 | 187 | 582 | 215 | 164 |
| 411 | 2210 | 2025 | 1987 | 1159 | 210 | 618 | 214 | 166 |
| 426 | 2043 | 1822 | 1780 | 980 | 210 | 590 | 163 | 132 |
| 429 | 2414 | 2217 | 2182 | 1261 | 238 | 683 | 209 | 163 |
| 430 | 2330 | 2133 | 2085 | 1201 | 266 | 618 | 193 | 179 |
| 432 | 1926 | 1716 | 1686 | 992 | 188 | 506 | 187 | 170 |
| Average | 2185 ±276 | 2004 ±256 | 1949 ±254 | 1115 ±106 | 220 ±28 | 614 ±76 | 213 ±41 | 153·25 ±17·51 |

Average percentage composition of carcass:

| | | |
|---|---|---|
| Average percentage composition of carcass: | Feathers | 8·3 |
| | Gut content | 2·5 |
| | Flesh | 51·0 |
| | Bones | 28·2 |
| | Offal | 10·0 |

Table II. *Percentage composition of the fresh material*

| No. of bird | *Flesh and offal* Moisture | Fat | Protein | Ash | *Bones* Moisture | Fat | Protein | Ash | *Total carcass* Moisture | Fat | Protein | Ash |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 305 | 74·95 | 4·04 | 20·14 | 1·06 | 55·95 | 8·63 | 21·61 | 12·96 | 69·07 | 5·47 | 20·59 | 4·77 |
| 310 | 75·18 | 4·52 | 19·34 | 1·13 | 58·60 | 9·14 | 19·05 | 12·17 | 70·15 | 5·93 | 19·25 | 4·48 |
| 312 | 73·31 | 6·11 | 19·33 | 1·01 | 57·78 | 9·53 | 19·59 | 10·88 | 68·01 | 7·27 | 19·42 | 4·37 |
| 313 | 78·32 | 2·06 | 18·91 | 1·04 | 63·12 | 4·04 | 20·52 | 12·06 | 73·30 | 2·72 | 19·44 | 4·69 |
| 314 | 75·56 | 3·81 | 19·78 | 1·00 | 59·58 | 8·81 | 19·91 | 11·25 | 70·42 | 5·43 | 19·83 | 4·32 |
| 315 | 74·57 | 5·95 | 18·56 | 1·11 | 57·75 | 12·47 | 18·01 | 11·21 | 69·18 | 8·02 | 18·38 | 4·33 |
| 321 | 72·26 | 7·29 | 19·58 | 1·02 | 56·09 | 12·31 | 18·99 | 12·23 | 67·46 | 8·78 | 19·41 | 4·35 |
| 322 | 73·75 | 6·23 | 19·37 | 1·12 | 56·67 | 11·79 | 19·36 | 11·58 | 68·87 | 7·83 | 19·38 | 4·13 |
| 323 | 73·19 | 6·54 | 19·36 | 0·98 | 55·83 | 10·55 | 19·93 | 12·89 | 67·69 | 7·82 | 19·54 | 4·76 |
| 326 | 69·44 | 8·63 | 20·58 | 1·12 | 57·10 | 11·11 | 19·20 | 10·96 | 65·64 | 9·40 | 20·16 | 4·16 |
| 328 | 79·20 | 1·44 | 18·77 | 0·97 | 61·89 | 5·41 | 20·85 | 11·59 | 72·90 | 2·88 | 19·53 | 4·83 |
| 329 | 74·57 | 4·94 | 19·58 | 1·06 | 56·76 | 9·65 | 20·95 | 12·53 | 69·50 | 6·28 | 19·97 | 4·34 |
| 331 | 74·40 | 5·08 | 19·82 | 1·01 | 59·25 | 10·32 | 18·91 | 10·86 | 69·63 | 6·72 | 19·50 | 4·09 |
| 332 | 74·76 | 4·97 | 19·03 | 1·01 | 57·45 | 10·06 | 19·38 | 11·19 | 69·55 | 6·50 | 19·14 | 4·07 |
| 337 | 76·21 | 3·70 | 19·21 | 1·16 | 59·24 | 10·02 | 19·05 | 10·74 | 70·50 | 5·83 | 19·15 | 4·38 |
| 342 | 76·22 | 4·30 | 19·08 | 0·99 | 59·09 | 10·82 | 18·32 | 10·81 | 70·51 | 6·44 | 18·84 | 4·21 |
| 343 | 76·61 | 3·71 | 19·22 | 1·00 | 59·92 | 7·55 | 19·88 | 12·37 | 71·22 | 4·94 | 19·43 | 4·64 |
| 347 | 69·24 | 10·89 | 18·38 | 0·94 | 53·90 | 14·80 | 18·54 | 11·82 | 64·74 | 12·04 | 18·42 | 4·07 |
| 348 | 69·39 | 9·99 | 19·84 | 1·00 | 54·67 | 14·51 | 19·72 | 10·85 | 64·94 | 11·29 | 19·66 | 4·04 |
| 350 | 71·71 | 8·32 | 18·99 | 0·98 | 56·31 | 13·89 | 18·96 | 10·43 | 66·56 | 10·13 | 18·98 | 4·15 |
| 354 | 74·22 | 5·41 | 19·08 | 1·05 | 56·03 | 11·64 | 18·81 | 12·34 | 68·25 | 7·45 | 19·00 | 4·74 |
| 355 | 76·03 | 4·20 | 18·89 | 1·03 | 59·33 | 10·21 | 18·21 | 11·09 | 70·52 | 6·20 | 18·66 | 4·36 |
| 358 | 75·30 | 5·18 | 19·01 | 1·00 | 58·03 | 11·33 | 18·43 | 11·19 | 69·90 | 7·10 | 18·83 | 4·17 |
| 359 | 73·81 | 6·03 | 19·36 | 1·04 | 57·77 | 12·14 | 19·33 | 10·17 | 68·61 | 8·02 | 19·36 | 4·01 |
| 364 | 69·00 | 11·19 | 18·76 | 1·15 | 58·03 | 11·60 | 19·61 | 10·35 | 65·80 | 11·31 | 19·01 | 3·88 |
| 369 | 74·47 | 4·60 | 19·71 | 1·13 | 59·36 | 9·95 | 19·86 | 10·53 | 69·66 | 6·31 | 19·76 | 4·12 |
| 370 | 74·61 | 3·76 | 20·46 | 1·08 | 58·28 | 9·53 | 19·33 | 11·38 | 69·60 | 5·54 | 20·01 | 4·26 |
| 375 | 76·86 | 3·05 | 19·33 | 0·99 | 61·71 | 6·38 | 19·50 | 11·52 | 71·99 | 4·12 | 19·32 | 4·37 |
| 376 | 75·01 | 5·14 | 18·90 | 1·12 | 57·19 | 10·82 | 19·81 | 11·09 | 69·13 | 7·01 | 19·20 | 4·39 |
| 385 | 75·60 | 2·61 | 20·23 | 1·19 | 58·20 | 7·98 | 20·00 | 13·24 | 70·25 | 4·27 | 20·16 | 4·90 |
| 386 | 72·11 | 6·32 | 20·95 | 1·61 | 56·51 | 9·75 | 20·65 | 12·44 | 67·17 | 7·40 | 20·84 | 4·61 |
| 391 | 74·09 | 5·73 | 19·38 | 1·01 | 56·79 | 11·55 | 20·05 | 11·44 | 68·53 | 7·60 | 19·60 | 4·36 |
| 393 | 74·54 | 5·25 | 19·39 | 1·12 | 59·80 | 9·29 | 18·41 | 11·76 | 69·90 | 6·52 | 19·07 | 4·50 |
| 394 | 73·89 | 5·83 | 19·24 | 1·03 | 54·17 | 11·94 | 21·26 | 12·40 | 67·54 | 7·80 | 19·89 | 4·70 |
| 404 | 72·04 | 7·15 | 19·63 | 1·00 | 54·95 | 11·80 | 19·88 | 11·98 | 66·62 | 8·62 | 19·71 | 4·48 |
| 411 | 72·35 | 7·45 | 18·79 | 0·99 | 56·05 | 12·49 | 18·33 | 11·69 | 67·28 | 9·02 | 18·65 | 4·31 |
| 426 | 75·88 | 3·28 | 19·54 | 1·06 | 60·88 | 9·11 | 18·44 | 10·52 | 70·91 | 5·21 | 19·18 | 4·20 |
| 429 | 72·64 | 6·12 | 19·67 | 1·09 | 55·84 | 11·99 | 19·92 | 11·32 | 67·36 | 7·96 | 19·74 | 4·29 |
| 430 | 69·53 | 10·46 | 18·56 | 1·00 | 57·43 | 10·79 | 19·33 | 11·37 | 65·94 | 10·55 | 18·78 | 4·08 |
| 432 | 71·25 | 8·36 | 18·92 | 1·01 | 55·19 | 13·65 | 18·61 | 11·61 | 66·42 | 9·92 | 18·82 | 4·19 |
| Average | 73·9 ±2·41 | 5·74 ±2·33 | 19·37 ±0·56 | 1·04 ±·061 | 57·71 ±2·11 | 10·49 ±2·21 | 19·46 ±0·86 | 11·52 ±0·76 | 68·79 ±2·06 | 7·24 ±2·11 | 19·39 ±0·54 | 4·35 ±0· |

*The nature of the variability in individuals of the same age, breed and sex*

The weight data given in Table I illustrate the fact that the growth rates of individuals of the same age and sex, and under identical conditions of feeding and management show considerable variation. This is in accordance with practical experience, but deserves special mention as emphasizing the fact that the weight of an individual is not necessarily an indication of age. The need for emphasizing this point is because of

the possibility of research workers selecting from among large flocks of individuals of mixed ages on experimental farms individuals of the same weight class as representing birds of the same age.

From inspection of the percentage composition data in Table II it at once becomes apparent that the chemical constituent of great variability is the fat.

*Analysis of the data*

In analysing the data presented, it was decided that the best method of attack was by means of scatter diagrams, as by such means the variation in the analyses given, and the possible correlations that may exist between the different items given in the analyses and weights, would be shown in pictorial form, and be thus more readily interpreted.

*Correlation between the fat content of the carcass and the moisture content*

The moisture content of adipose tissue is low as compared with that of flesh and offal, it would therefore be expected that as the fat content

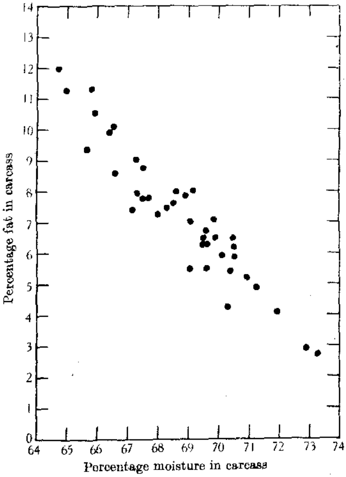


Fig. 1.

of the carcass increases the moisture content should fall. The percentage fat figures of the carcass were therefore plotted against the percentage moisture figures (see Fig. 1). It will be noted that this expectation is fully corroborated by the figures plotted, the moisture content showing a well-defined decrease as the fat content increases. Statistical treatment of the data gave a coefficient of correlation of the variables percentage moisture and percentage fat of $-0{\cdot}9480 \pm 0{\cdot}108$, thus showing a strong negative correlation between these two variables.

*Correlation between live-weight and fat content*

In the majority of fattening trials, the live-weight increase is taken as a measure of the efficiency of a ration, and the relative efficiencies of

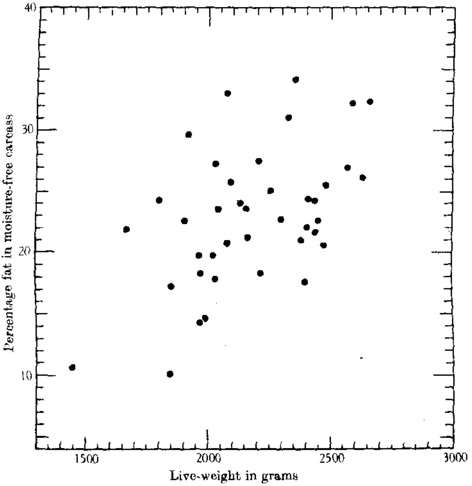


Fig. 2.

different foods are measured according to their efficiency in producing this live-weight increase. This measurement of efficiency would be justified if it could be established that there is a direct relationship between

the live-weight of birds of the same age and the fat content of the carcasses. The fat percentages in the carcasses of the birds analysed were therefore plotted against the live-weights to ascertain whether such a correlation does in fact exist (see Fig. 2).

It will be noted that there is a slight trend shown for the fat content of the carcass to increase as the live-weight increases, but the scatter between individuals of approximately the same live-weight is so great that it is obvious that attempts to measure the energy storage in animals by consideration of live-weight increases are of doubtful value, and would involve the employment of abnormally large groups of individuals. In view, however, of the trend shown for heavy individuals of the same age class to have a larger proportional energy content than light individuals, the data have been submitted to detailed statistical analysis to ascertain whether this trend is statistically significant (see p. 390).

*Correlation between the fat content of the bones and that of the flesh and offal*

The fat present in the body fulfils two functions, it plays an essential role in the metabolic processes, it also acts as a reserve store of energy. Inspection of the data given in the percentage composition of fresh material table reveals the fact that fat is a constant element in the composition of bone. On the assumption that the fat so present plays an essential part in the fat metabolism of the body, and bearing in mind the established fact that the reserve fat depots of the body are situated in the soft tissues of the body, it would be expected that the fat content of the bones would be correlated with the fat content of the flesh and offal, but that this correlation would not be so marked in the case of the carcasses with high fat content. Fig. 3 has been constructed by plotting the fat percentages in the moisture-free flesh and offal against those of the moisture-free bones. It will be noted that there is a general correlation between the fat content of the bones and that of the flesh and offal, the fat content of the bones increasing as the fat content of the flesh and offal increases. It will further be noticed that the increase in the fat in the flesh and offal is steeper than that in the fat of the bones, a fact in accordance with the hypothesis stated above. It can therefore be stated that the bones play an important part in the general fat metabolism of the body, but that the fat reserve depots are essentially present in the soft tissues of the body. This fact is brought out quite clearly if we

group together the actual amounts of fat present in the bones and flesh and offal respectively.

| No. of cases in which the fat in flesh and offal lie between | | Of these the fat in the bones exceeds that in the flesh and offal in |
|---|---|---|
| 10 and 40 g. | 5 | 3 cases |
| 41 and 70 g. | 13 | 7 cases |
| 71 and 100 g. | 14 | 1 case |
| 101 and 140 g. | 4 | None |
| 141 and 170 g. | 4 | None |

In other words, in the case of a bird with low total fat content, the fat in the bones may exceed that in the flesh and offal, but in the case of

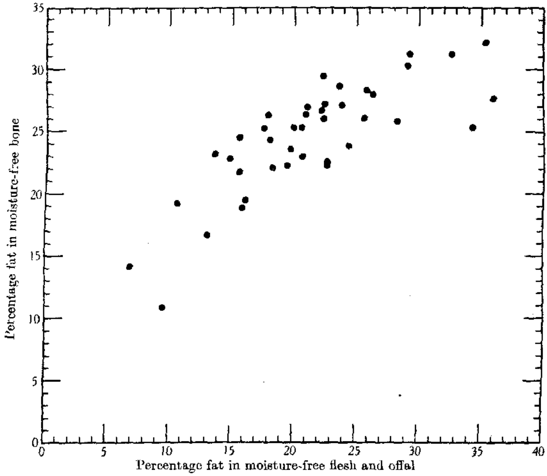


Fig. 3.

a well-fatted bird in which the reserve store fat is well represented, the total fat in the bones is invariably less than that in the flesh and offal.

*Correlation between the ash in the bones and that in the flesh and offal*

In another communication, it has been demonstrated that whereas during growth the percentage ash of the flesh of the bird remains approximately constant that in the bones increases with age. Moreover,

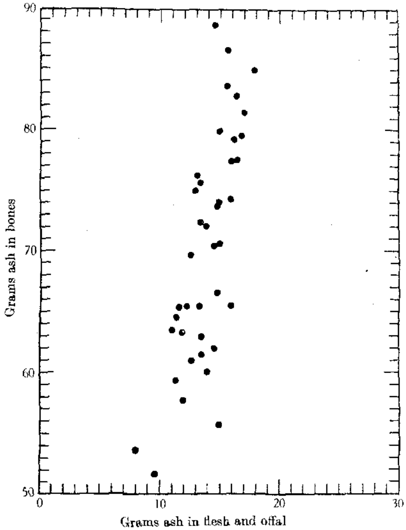


Fig. 4.

the composition of the ash of the flesh is markedly different from that of the bones, the bone ash being characterized by a high proportion of calcium and phosphorus, whereas the ash of the flesh is characterized by a high proportion of potassium and sulphur. This difference in composition is, of course, a reflex of the fact that the metabolic functions of the ash of the flesh are different from those of the bone ash; any correlation, therefore, that may be shown between the bone ash and the

ash of the flesh and offal must find an explanation in some other direction than similarity of function.

If the amounts of ash present in the flesh and offal are plotted against those present in the bones it will be noted that a correlation does exist, the ash of the bones representing about five-sixths of the total ash in the carcass (see Fig. 4). In view of the fact that the birds varied in live-weight from 1453 to 2666 g. the parallelism that exists between the bone ash and the flesh and offal ash indicates that the skeletal structures and the soft tissues developed in the same ratio, for all the birds analysed, i.e. the slowing or quickening of the growth impulse that resulted in the production of a small or a big bird evidently affected the growth of flesh and bone proportionately. The correlation coefficient $r = +0{\cdot}7392 \pm 0{\cdot}0438$, showing a high positive correlation between the ash in bones and the ash in the flesh and offal.

### *Correlation between the protein and ash*

Assuming the correctness of the explanation given above of the parallelism shown between the bone ash and the flesh and offal ash, we should expect to find a close correlation between the protein of the carcass

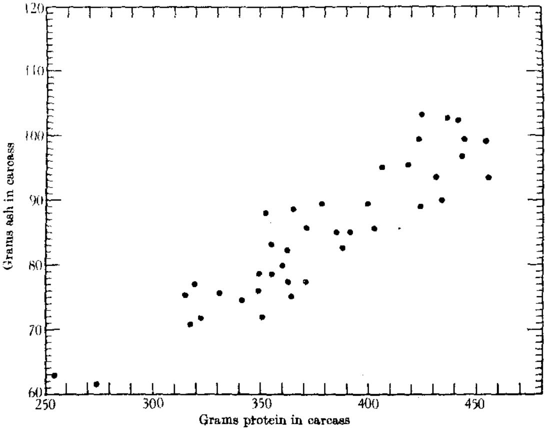


Fig. 5.

and the ash. In view of the variability in the fat and moisture content of the samples analysed, a true correlation between the ash and the protein can best be obtained by plotting the protein in grams in the carcass against the ash in grams in the carcass (see Fig. 5).

Inspection of this diagram will show that the correlation is quite good, and we can accept with some reliance the proposition that the growth of

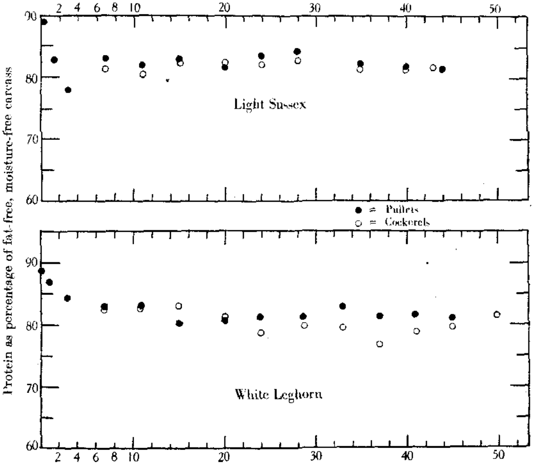


Fig. 6.

the flesh in an individual and the growth of the bone are not independent but are linked together and actuated by the same growth impulse or "wachstumstrieb". The differences met with in the early stages of growth are not due to differential growth rates, but are really a reflex of the slowness of calcification in the developing bone structures. In view of this close correlation between the protein and ash of birds of the same breed and age but of varying live-weights, it was decided that it would be of interest to ascertain to what extent this correlation is shown during growth. For this purpose, analyses of pullets and cockerels of

the White Leghorn breed and the Light Sussex breed of different ages, obtained in a previous experiment, were plotted on a similar basis. Each analysis represented the average of three birds, except in the earlier stages of the experiment, when six birds were used as a sample (see Fig. 6). From the diagram it will be noted that the main changes in the ratio of protein to ash occur in the first seven weeks of the chicks life, both in the case of the Light Sussex and the White Leghorn, and that the protein ash ratio remains fairly steady thereafter. There is also a tendency for the ash in the cockerels to be higher than that in the pullets, doubtless reflecting the sex difference in bodily structure. The White Leghorn breed also shows a lower protein and a higher ash percentage than the Light Sussex, this is in accordance with the difference to be expected between a typical egg producing breed and a typical meat producing breed.

*Correlation between the live-weight and the energy content per unit live-weight (Cal. per 100 g.)*

It has already been shown that the carcasses of the forty cockerels analysed varied considerably in their fat content, and that there was no very apparent correlation between the live-weight and the fat content for birds of different weights. In view of its obvious importance, it was decided to study this aspect of composition in more detail, and for this purpose the energy content per 100 g. of live-weight was calculated, using the factors 5·34 Cal. per g. for protein, and 9·43 Cal. per g. for fat (see Table I).

Applying the formula

$$r_{xy}=\frac{\dfrac{\Sigma\,(xy)}{n}-(\bar{x}\bar{y})}{\sqrt{\left(\dfrac{\Sigma\,(x^2)}{n}-(\bar{x})^2\right)}\sqrt{\left(\dfrac{\Sigma\,(y^2)}{n}-(\bar{y})^2\right)}},$$

where $x$ = live-weight in kg. and $y$ = Cal. per 100 g. live-weight and $n$ = no. of observations, $r_{xy}=+0{\cdot}5077$.

The probable error of the coefficient of correlation,

$$Er_{xy}=\frac{\pm 0{\cdot}6745\,(1-r^2)}{\sqrt{n}},$$

worked out at $\pm 0{\cdot}0792$, whence $r_{xy}=+0{\cdot}5077\pm 0{\cdot}0792$, indicating a high positive correlation between live-weight and energy content. By interpolation from Fisher's table V A with $P=0{\cdot}01$ and 38 degrees of freedom

$r=0\cdot4032$. The correlation is therefore statistically significant, since the $r$ obtained in the experiment (0·5077) is considerably greater than that required for a $P$ of 0·01. We can therefore regard as statistically significant the tendency of heavy individuals of the same age class in cockerels to have a higher caloric content per 100 g. of live-weight than light individuals.

In carrying out experiments by the slaughter method it will consequently be more advantageous to select from the same age class individuals of approximately equal live-weights rather than to take a random sample of individuals of widely varying live-weights.

*The variability of caloric content per* 100 *g. live-weight*

Inspection of Table I reveals the fact that the energy or caloric content per 100 g. of live-weight of the individuals analysed varies from 115 Cal. to 260, with an average value of 153·25 Cal. By statistical treatment it can be shown that the standard deviation is $\pm 17\cdot51$ Cal. The coefficient of variation consequently becomes $\frac{\pm 17\cdot51 \times 100}{153\cdot25}\ \% = \pm 11\cdot42\ \%$. Since the probable error of the mean of a sample is $0\cdot6745\ \frac{\sigma}{\sqrt{N}}$ the number of birds to be included in a sample to give a representative analysis of a similar group of the same age weight class can be calculated. It thus becomes possible to indicate the number of birds that should be analysed and to predict the extent to which this analysis is representative of the surviving groups of the same age class.

| No. of birds | Limits of accuracy of energy content % |
|---|---|
| 55 | ±1 |
| 14 | ±2 |
| 6 | ±3 |
| 3 | ±4 |
| 2 | ±5 |

In spite, therefore, of the known variability in the fat content of individual birds, the slaughter group method is practicable, since fourteen birds in a group will suffice to give a measure of the energy content of the group within a variation of $\pm 2\ \%$.

## Summary

Individual analyses of forty Light Sussex cockerels of twenty-two weeks of age yielded the following results:

1. *There is a highly negative correlation between the fat percentage* of the carcass and the moisture percentage.

2. Owing to the large variability in the fat content of individuals, live-weight increases are unreliable as a measure of energy storage.

3. Comparison of the fat content of the bones with that of the flesh and offal leads to the conclusion that the bones as well as the flesh and offal play a significant role in the general fat metabolism of the body.

4. *There exists a strongly positive correlation between the ash* content of the bones and that of the flesh and offal.

5. The protein ash ratio of both the Light Sussex breed and the White Leghorn breed is practically constant for all individuals after the age of seven weeks, indicating that the growth of the skeletal and the muscular tissues are closely correlated and controlled by the same growth impulse.

6. The *individual* variation *in* energy *content* is *shown* to be ± 11·42 % of the total energy content.



## Acknowledgement

The author desires to record his appreciation of the services rendered by his assistant, Mr C. J. L. Baker, during the course of this investigation, in particular for the care shown in the preparation and analysis of the experimental material.


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# THE IGNITION AT LOW TEMPERATURES OF THE ORGANIC MATTER IN SOILS

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(With Three Text-figures)

## Introduction

The prior removal of organic matter frequently facilitates the subsequent chemical examination of soils and, in the case of the determination of trace metals, by spectrography, or purely chemical methods, is always advisable. The possibility of either solution losses or the introduction of small quantities of the sought-for metals as impurities in the reagents employed, renders wet methods of oxidation unsatisfactory. The ignition of the soil, while not suffering from the latter disadvantage may, however, cause the loss of certain relatively volatile compounds of such metals as zinc or cadmium. In order to determine the rate of removal of organic matter from soils at much lower temperatures than are usually employed in ignition, the following investigation was carried out.

## Experimental technique

Five soils, four Australian and one English, together with a sample of Merck's "humic acid" and a sample of cellulose (filter paper), relevant analytical data for which are given in Table I, were examined.

Table I. *Analytical data for the soils, humic acid and cellulose used in the investigation*

| Soil no. | 4303 | 90A | 3496 | 1518 | 2719 | Humic acid | Cellulose |
|---|---|---|---|---|---|---|---|
| Soil type | Podsol | Volcanic ash | Red-brown earth | Swamp | Fen | — | — |
| | % | % | % | % | % | % | % |
| Coarse sand | 23·1 | 20·9 | 2·1 | 0·2 | 0·8 | — | — |
| Fine sand | 53·4 | 46·4 | 44·7 | 7·5 | 4·4 | 0·67* | 0·01* |
| Silt | 5·1 | 10·0 | 30·8 | 24·5 | 10·1 | — | — |
| Clay | 6·2 | 7·2 | 17·4 | 54·1 | 22·8 | — | — |
| Loss at 100° C. | 2·55 | 3·36 | 1·80 | 7·39 | 18·87 | 7·00 | 8·42 |
| Loss at 900° C. | 11·06 | 8·33 | 4·73 | 12·93 | 46·43 | 92·33 | 91·57 |
| Organic carbon† | 5·73 | 4·77 | 1·40 | 4·13 | 24·40 | 59·6 | 40·68 |
| Organic nitrogen | 0·13 | 0·38 | 0·12 | 0·46 | 1·68 | 3·12 | — |
| Carbon: nitrogen ratio | 43·1 | 12·5 | 12·2 | 9·0 | 14·5 | 19·1 | — |

* Ash. † Determined by dry combustion.

[1] Division of Soils, Council for Scientific and Industrial Research.

The organic matter of the soils varied in composition from a mixture of undecomposed woody and humified material, in soil no. 4303, a surface sample of a well-developed podsol, to well-humified, in soil no. 1518, a typical swamp soil from the Murray River areas. In their organic matter content the range was from 2·5 % in soil no. 3496, a red-brown earth from the Waite Institute, to over 40 % in soil no. 2719, a fen soil from Cambridge, England. The soils were also chosen to represent a range of texture classes, from sands to a heavy clay.

Soil samples were weighed into shallow 10 cm. porcelain combustion

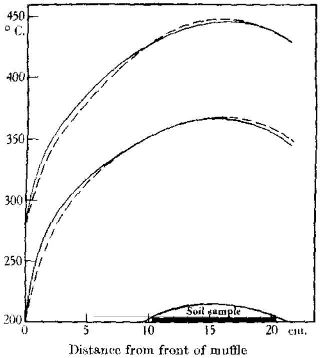


Fig. 1. Showing temperature variations 0·5 cm. above the floor from the front to the back of muffle and position of soil sample during ignition. (Full line for the centre, broken line for the corners.)

boats, and after drying in an oven overnight, were ignited in an electric muffle at temperatures ranging from 200 to 500° C. for periods of from 2 hours to 1 week. Following ignition, the soils were transferred to Erlenmeyer flasks, and the residual organic carbon determined by wet oxidation (Walkley, 1935). The carbon in the original soil was determined by both wet and dry combustion, the figures obtained by the latter method being those given in Table I. While the results obtained by the former method are always low, the results following ignition are proportional, and percentage losses may be satisfactorily calculated from the wet-combustion figures obtained for the soil itself.

A survey of the temperatures in the different parts of the muffles

employed showed that, while there was little variation within any vertical plane parallel to the front of the muffle, there was a marked range from front to back. The maximum temperatures in the lower half of the muffle occurred at a distance of about 15 cm. from the front. On either side of this plane for a distance of 5 cm. the temperatures varied by only 10° C., but in the forward 10 cm. there was a rapid fall of over 100° C., when the maximum temperatures recorded were between 300 and 500° C. Temperature gradients for a height of 0·5 cm. above the floor of the muffle are shown in Fig. 1.

For the ignition of the soils only the hottest part of the muffles were used; the 10 cm. boats containing the soil were placed longitudinally on the floor of the muffle, with their centres in the plane of maximum temperature (see Fig. 1). Ten samples representing five soils in duplicate could be ignited at one time and, in order to ensure comparable heating of each soil, the duplicates were arranged in reverse order on either side of the thermometer at the centre.

Table II. *Percentage losses of organic carbon from soils and humic acid heated for periods at various temperatures*

| Period of ignition hr. | Temperature of ignition ° C. | Soils | | | | | | Humic acid |
|---|---|---|---|---|---|---|---|---|
| | | 4303 % | 90 A % | 3496 % | 1518 % | 2719 % | Mean % | % |
| 2 | 210 | 14·4 | 15·8 | 11·7 | 18·9 | 10·0 | 14·1 | — |
| | 260 | 39·1 | 45·4 | 36·9 | 43·8 | 41·8 | 41·4 | — |
| | 310 | 57·9 | 69·7 | 60·2 | 69·0 | 72·8 | 65·9 | — |
| | 350 | 81·5 | 81·9 | 80·9 | 78·8 | 81·3 | 80·9 | — |
| | 400 | 95·3 | 91·9 | 89·8 | 89·0 | — | 91·5 | 96·54 |
| | 450 | 99·5 | 99·1 | 98·9 | 98·4 | — | 99·0 | 100·0 |
| | 500 | 99·93 | 99·75 | 99·83 | 99·75 | 99·89 | 99·83 | 100·0 |
| 4 | 210 | 22·2 | 24·0 | 20·0 | 27·5 | 20·8 | 22·9 | — |
| | 260 | 44·0 | 55·4 | 45·1 | 51·6 | 55·8 | 50·4 | — |
| | 310 | 66·6 | 76·2 | 70·6 | 76·9 | 78·0 | 73·7 | — |
| | 350 | 89·5 | 87·6 | 85·5 | 83·2 | 85·3 | 86·2 | — |
| | 395 | 98·1 | 95·7 | 96·0 | 92·6 | — | 95·6 | 99·7 |
| | 460 | 99·80 | 99·6 | 99·6 | 99·6 | — | 99·65 | 100·0 |
| | 500 | 99·98 | 99·95 | 99·94 | 99·95 | 99·94 | 99·95 | 100·0 |
| 8 | 210 | 25·9 | 29·4 | 24·0 | 32·2 | 28·0 | 27·9 | — |
| | 260 | 49·8 | 63·9 | 53·4 | 61·5 | 68·5 | 59·4 | — |
| | 310 | 74·8 | 80·6 | 75·4 | 79·4 | 80·9 | 78·2 | — |
| | 355 | 92·5 | 90·6 | 88·9 | 86·2 | 88·1 | 89·3 | — |
| | 395 | 98·8 | 97·1 | 97·3 | 94·5 | — | 96·9 | 99·94 |
| | 460 | 99·94 | 99·78 | 99·86 | 99·78 | — | 99·84 | 100·0 |

The muffle was closed by means of a fairly loose fitting asbestos door with three small holes, one at the centre and one at each corner, 0·5 cm. above the floor. These holes served either to insert the thermometers or as inlets for air.

The stems of the thermometers were so calibrated that the temperature at any point along the length of the combustion boats could be read directly. During ignition the temperature at the maximum position was recorded at regular intervals, while the range over the length of the boats was taken less frequently. By means of variable resistances placed in the electrical circuits the maximum temperatures could be maintained within about 5° C. for extended periods. The temperature of ignition for any period was taken as the mean temperature, to the nearest 5° C., of the whole soil during the determination.

Losses at 100 and 150° C. were obtained by heating the soils in a regulated electric oven. To prevent decrepitation during ignition all soils were dried at 100° C., and, while losses of organic matter at this temperature were appreciable, they were considered to have little if any effect on the final resultant losses of the subsequent ignitions.

Table III. *Percentage losses of organic carbon from soils, humic acid, and cellulose, heated for periods up to 1 week at various temperatures*

| Period of ignition hr. | Temperature of ignition ° C. | Soils 4303 % | 90 A % | 3496 % | 1518 % | 2719 % | Mean % | Humic acid | Cellulose |
|---|---|---|---|---|---|---|---|---|---|
| 16 | 100 | 5·7 | 2·5 | 3·3 | 4·7 | 1·9 | 3·6 | 5·9 | 0·3 |
| | 150 | 9·9 | 5·8 | 5·1 | 9·0 | 3·3 | 6·6 | 12·0 | 1·1 |
| | 210 | 36·0 | 38·3 | 32·8 | 42·2 | 33·5* | 37·2 | 34·5 | 3·9* |
| | 255 | 49·8 | 66·5 | 57·1 | 65·0 | 74·0† | 61·7 | 62·1 | 73·3† |
| | 310 | 89·6 | 88·1 | 86·6 | 87·0 | 85·3‡ | 87·7 | 89·5 | 81·7‡ |
| | 345 | 97·1 | 94·8 | 95·6 | 91·3 | 94·1§ | 94·5 | 99·6 | 90·0§ |
| | 395 | 99·5 | 98·6 | 99·0 | 97·8 | 99·26¶ | 98·8 | 100·0 | 100·0¶ |
| | 460 | 99·97 | 99·88 | 99·93 | 99·88 | 99·97 | 99·93 | 100·0 | 100·0 |
| | 500 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |
| 32 | 210 | 38·8 | 48·9 | 39·6 | 48·5 | 44·0 | 44·0 | — | — |
| | 265 | 60·2 | 74·5 | 65·8 | 74·9 | 78·3 | 70·7 | — | — |
| | 310 | 91·1 | 89·5 | 88·7 | 87·6 | 88·4 | 89·1 | — | — |
| | 355 | 98·5 | 96·4 | 97·5 | 93·7 | 95·8 | 96·4 | — | — |
| 72 | 215 | 46·0 | 60·8 | 50·6 | 58·5 | 64·6 | 56·1 | — | — |
| | 265 | 73·0 | 81·8 | 77·1 | 83·3 | 83·2 | 79·7 | — | — |
| | 310 | 95·0 | 92·6 | 90·8 | 90·3 | 91·6 | 92·1 | — | — |
| | 355 | 99·2 | 97·5 | 98·4 | 96·2 | 97·3 | 97·7 | — | — |
| 168 | 210 | 48·4 | 65·2 | 53·9 | 63·8 | 70·2 | 60·3 | — | — |
| | 265 | 81·2 | 84·3 | 80·9 | 86·4 | 86·2 | 82·6 | — | — |
| | 310 | 98·0 | 95·0 | 96·0 | 93·9 | 94·2 | 95·4 | — | — |
| | 355 | 99·7 | 98·8 | 99·4 | 98·6 | 98·6 | 99·0 | — | — |

* 205° C. † 260° C. ‡ 305° C. § 350° C. ¶ 400° C.

## Discussion of results

While all individual results are tabulated in Tables II and III, the percentage losses of organic matter from each soil, the humic acid and the cellulose after 16 hr. (overnight) heating, at temperatures ranging

up to 500° C. are shown in Fig. 2. In Fig. 3 the mean rate of loss of organic carbon from the five soils is shown for specified temperatures of ignition.

An examination of Fig. 2 indicates that the loss of organic matter at temperatures as low as 100° C. may be quite appreciable, and that the so-called "moisture" figures therefore include a proportion of organic matter. The 7 % of the humic acid lost at 100° C. contained 35 % of organic carbon.

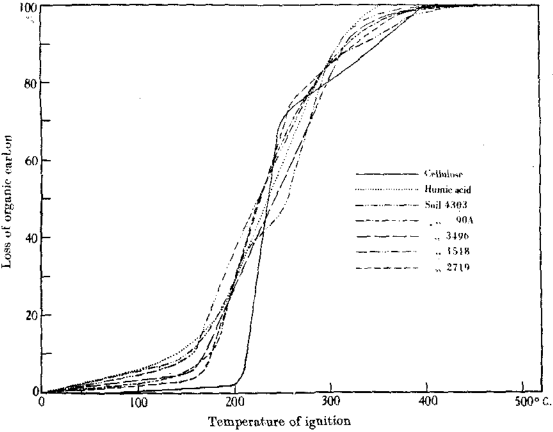


Fig. 2. Showing the percentage losses of organic carbon after 16 hr. ignition at temperatures ranging up to 500° C.

Below 200° C. losses are due essentially to the distillation of the volatile constituents of the organic matter. Between 200 and 300° C., while ordinary distillation continues, the main reaction is one of destructive distillation with carbonization of the residue. Above 300° C. the reaction is principally the ignition of this carbonaceous material.

Only small losses are sustained by the cellulose below 200° C., although slow carbonization is evident in the brown colour developed in the material at this temperature. With a rise in temperature, the cellulose suffers rapid destructive distillation, and at 260° C. the residue consists

entirely of carbon. A temperature of 400° C. is necessary for complete ignition of this carbon with a period of ignition of 16 hr.

The humic acid, which contains no cellulose but is composed principally of protein and ligneous material, shows 40 % of volatile constituents below 200° C. Although destructive distillation commences at this temperature, the destruction of the humic acid is by no means as rapid as that of cellulose and carbonization is not apparent even at 300° C., since the percentage losses at this temperature, both for organic carbon and the organic matter as a whole as shown by loss on ignition, are almost identical. Above 300° C. a little charring, as evidenced by a change in colour from brown to very dark brown or black, takes place just prior to the complete ignition at about 360° C.

The proportion of organic matter, volatile below 200° C., is much the same in all soils as for the humic acid, although there is a marked variation in the volatility of the constituents of each soil. Above 200° C. the soils in which the organic matter is well humified show similar rates of loss to that of the humic acid itself, but in the case of the sandy soil containing woody and undecomposed fibrous material, a slowing up in the rate is apparent over the 200–260° C. range.

By 300° C. approximately 85 % of the organic matter of the soils and the humus is removed. The effect of the inorganic material of the soil and its texture in reducing the rate of removal of the remaining small fraction of the organic matter in the soil, becomes appreciable at this point. In the case of the cellulose and humic acid the oxygen of the air is in close contact during the whole course of the ignition. With the soils, however, the mineral matter reduces the free access of oxygen, and consequently the removal of the last traces of carbon is governed to a certain extent by the diffusion of the carbon dioxide out of, and the oxygen into the soil mass. Under conditions of oxygen restriction a greater degree of carbonization takes place than with the completely exposed humic acid, with a result that the rate of ignition of the organic matter in the soil over the range 300–400° C. is intermediate between that of the humic acid and the cellulose. Gaseous diffusion within the sandy soils is less restricted than in the heavier soils, and the losses from soil no. 4303 are much higher at the higher temperatures than from soil no. 1518. In the final stages of ignition the limits placed upon the rate of supply of oxygen prolong the burning of the soil carbon beyond that temperature at which the free carbon from the cellulose disappears. With a period of 16 hr. ignition, a temperature of 500° C. is therefore necessary to remove the organic matter completely from the soil.

It must be understood that the supply of oxygen within the muffle itself is at no time a limiting factor. Samples of humic acid, cellulose and the fen soil, varying in weight from 0·5 to 5·0 g. heated singly or together for a period of 16 hr., showed differences in the percentage losses, at various temperatures, to be well within experimental error.

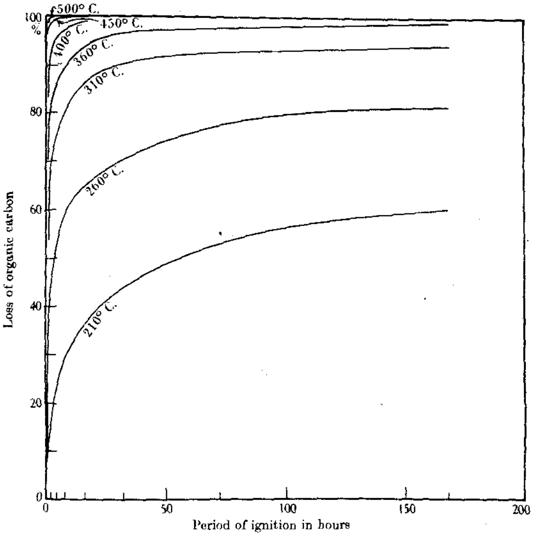


Fig. 3. Showing the mean percentage losses of organic carbon for specific temperatures for periods ranging up to 1 week.

In Fig. 3 are shown the mean percentage losses for the five soils examined, at certain specific temperatures for periods of ignition up to 1 week. At the lower temperatures, up to a period of 3 days, the course of the reaction is of a multimolecular—probably the fifth—order, although the rate slows down materially afterwards. With increasing temperature the order of the reaction decreases, and above 400° C. approaches that of a bimolecular one, over the periods for which losses were determined.

From the diagram it is possible to determine equivalent periods of heating necessary at any temperature to remove the same quantity of organic matter. For the removal of 99 % of the organic matter, a percentage which may be considered practicable for most purposes, 1 week at 350° C., 16 hours at 400° C., 2 hours at 450° C. or about half an hour at 500° C. are necessary.

## Summary and conclusions

*The low temperature ignition of soil organic matter has been investigated for temperatures ranging from 100 to 500° C.*

Appreciable losses are found to occur below 100° C.; up to 200° C. heating results essentially in the distillation of volatile constituents, while between this temperature and 300° C. destructive distillation is the major reaction. These reactions are responsible for the removal of 85 % of the soil organic matter.

By 300° C. the greater part of the residual organic matter consists of carbonaceous material, and the final reaction is simple ignition of this material.

Two hours' heating of the soil at 450° C. or about half an hour at 500° C. are recommended for the removal of 99 % of the soil organic matter.

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# THE EFFECT OF THE "GYROTILLER" ON CROP YIELD

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## Introduction

The first "Gyrotiller" was constructed in 1927, the early machines being used in tropical countries, and chiefly in preparing land for sugar cane. The general opinion of its work in those countries seems to have been favourable, and in particular large claims were advanced for it on the score of economy (Anon., 1932; Bell, 1932). Although the implement has always been made in this country, it was not until 1932 that it was available to British farmers; when the machine was first seen it was decided to lay out experiments on the Cambridge University Farm to test its capabilities.

Deep cultivation has been practised for some time, and the agricultural literature of the middle of the nineteenth century shows that the subject exercised the minds of practical men constantly from the time of the introduction of steam cultivation. Numerous experiments have been carried out to determine the effect of deep ploughing, but Sewell (1919), who reviewed the literature on the subject, concluded that "deep plowing (more than 7 in. deep), in general, does not increase crop yields". Since deep ploughing necessitates bringing subsoil to the surface, it is only to be expected that results in different situations would be very variable and would depend largely on the nature of the subsoil. But in increasing depth of cultivation it is unnecessary to bring unweathered material to the surface, and that is avoided by the usual method of subsoiling. This operation again has given variable results in experiments, though extensive trials laid down in Essex and Oxfordshire were generally favourable to it (Anon., 1923–6).

The "gyrotiller" has a very different action from that of any other implement, and gyrotilling resembles subsoiling rather than deep ploughing in that only a little unweathered material is brought to the surface; the disturbance of the subsoil, however, is very much greater in the case of the "gyrotiller", and it is possible to work to greater depths. The thorough movement of the soil to a depth of 12 in. or more must

obliterate any plough pans and, on sticky clay land, should assist drainage; soil studies on the experiments to be described below have shown that the breaking up of the subsoil has assisted drainage (Nicholson, 1934), and also that a difference in looseness in the soil can be detected for a long time after the operation (Culpin, 1936 *a*, *b*).

This paper is only concerned with the effect of the "gyrotiller" on crop yields, a subject on which as yet little work has been published. The opinion expressed after the first season's work of a "gyrotiller" in New Zealand was favourable, provided that the operation was carried out some time before the crop was planted (Stafford, 1936). Walker (1935), in Scotland, was impressed with the thoroughness of the machine's work, but stated that farmers' opinions as to its efficacy were divided. No precise experimental results have been published in this country, but an experiment with the "gyrotiller" was carried out at Jealott's Hill Agricultural Research Station in 1935 (Watson, 1935). In this experiment gyrotilling was compared with ploughing, and although the wheat on the gyrotilled plots looked more vigorous throughout the winter, it gave no higher yield than that on the ploughed plots.

The present paper describes four experiments, each of from two to four years' duration, carried out on the Cambridge University Farm, and concerns itself solely with the crops; a large number of soil studies has been made but the results of these have been, or are being, published by other workers. Two of the experiments (Girton Allotment and Longfallen) were on very heavy gault clay, and two (Bunkers and Dry Field) on light gravelly land which tends to form pans below the normal depth of ploughing.

### *Girton Allotment*

This field carried a crop of oats and tares which was taken for hay in June 1933. Farmyard manure was carted out, spread, and ploughed in by 15 July. Gyrotilling was done on 17 July. The field, of 13 acres, is approximately rectangular, with the long side running up an appreciable slope. The experiment consisted of six randomized blocks of two plots, each of which extended the full length (250 yd.) of the field. The plots were actually marked out by the "gyrotiller" and each consisted of four widths of working, varying in breadth from 40 to 44 ft.; the control plots were not traversed by the implement. The "gyrotiller" worked to a depth which varied from 16 to 18 in., and very little of the farmyard manure was brought to the surface; on the other hand a few very large clods of subsoil were brought to the top. One danger of this deep

working of the land is that drains may be disturbed, but in the present case there was no evidence that the mole drains or mains were damaged and, in fact, the gyrotilled land appeared subsequently to drain rather more freely than the control. The control plots were horse-cultivated on 19 July, after which the whole field was treated uniformly, a bastard fallow being taken during the remainder of the summer.

Throughout the period of the experiment the procedure was to restrict counts and weighings to strips across the plots, care being taken in marking out the actual plots to allow sufficient discard to cover any irregularities in the edges of the strips. For each experimental crop a different strip was taken, because other treatments were generally superimposed: it will, of course, be realized that a plurality of strips did not give an increase in replication, which was restricted to six throughout.

The first crop to follow the treatment was Wilhelmina wheat, drilled on 13 and 14 October, the gyrotilled plots having a somewhat coarser tilth. It was not thought at the time that there was any appreciable difference in depth of drilling, but the plants in the control plots came up slightly earlier, and were much thicker, and it is believed that on the gyrotilled plots some seed fell down crevices to a considerable depth. It is not uncommon to hear of cases where poor germination is obtained after gyrotilling, but it was surprising to find this occurring when there was an interval of three months between gyrotilling and drilling. Throughout the succeeding winter and early spring the sparseness of plants on the gyrotilled plots was very noticeable, and counts showed that those plots carried 10 % fewer plants than the non-gyrotilled plots, and that they started to tiller later in the spring. By the beginning of May a reversal had taken place, the gyrotilled plots then exhibiting a darker green appearance and being definitely more healthy: excavations made on 14 May showed a clear difference in root system, the plants on the gyrotilled plots having thicker, deeper and more extensive roots than those of the control plots. A thunderstorm occurred about a week before the crop was harvested, and this caused serious lodging on the non-gyrotilled plots, whereas the wheat on the gyrotilled plots stood well, the line of demarcation being clearly defined.

In this experiment two transverse strips were taken across the gyrotilled plots, the two strips being contiguous except for a pathway, 1 yd. wide, between them. One of the strips was 20 yd. broad and was reserved for the measurement of harvest yields; this provided main plots of approximately 1/24 acre, though discards between subplots (for a comparison of spring treatments (Garner & Sanders, 1937) reduced

the actual area harvested for a main plot. The strip on the other side of the pathway was only 10 yd. wide and was used for sampling; the arrangement of having separate areas for developmental studies and for harvest yield has little to recommend it, and has since been abandoned.

Table I A. *Girton Allotment experiment. Wheat, first crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| From samples | | | | | |
| *No. plants per ft. (2. i. 34)* | 13·62 | 15·30 | 0·290 | 89·0 | O>G** |
| *No. stems per ft. (2. iii. 34)* | 17·65 | 29·65 | 0·980 | 59·5 | O>G*** |
| *No. stems per ft. (12. iv. 34)* | 47·65 | 61·98 | 1·929 | 76·9 | O>G** |
| No. ears per ft. | 19·02 | 19·81 | 0·377 | 96·0 | Insignificant |
| Wt. grain per ft. corrected for no. of ears | 24·90 | 23·06 | 0·542 | 108·0 | Insignificant |
| Wt. grain per ft. corrected for no. of plants | 25·74 | 22·23 | 0·506 | 115·8 | Insignificant |
| From plots | | | | | |
| Wt. grain (bush./acre) | 58·39 | 56·64 | 1·148 | 103·1 | Insignificant |
| Wt. straw (cwt./acre) | 59·23 | 65·17 | 0·976 | 90·9 | O>G** |

*Note.* In this and subsequent tables:

* Denotes significance $P<0{\cdot}05$.
** ,, ,, $P<0{\cdot}01$.
*** ,, ,, $P<0{\cdot}001$.

The results for the crop are given in Table I A, where the decrease in plant number and stem number on the gyrotilled plots is shown to be highly significant, as also was the decreased yield of straw. As regards weight of grain the gyrotilled plots slightly out-yielded the controls, and when correction was made (on samples) for number of plants in January the difference amounted to nearly 16 %; the sacrifice of a degree of freedom in the analysis of covariance reduced the number in the error line to 4, and consequently this was also insignificant, but the indication that the plants on the gyrotilled plots grew better cannot be entirely ignored.

The following crop was Marvellous oats, and dynamometer tests carried out during the ploughing in autumn showed that the resistance on the gyrotilled plots was very much reduced (Culpin, 1936 *a*, *b*). The oats were drilled on 15 March, the main plots being split for a comparison of methods of applying artificial manure: there were three subplots, one receiving 1½ cwt. of sulphate of ammonia and 3 cwt. of superphosphate per acre drilled with the seed, one the same amount of manure broadcast over the surface, and the third no manure. It was decided to superimpose this manurial enquiry on the main plots in order to test the

suggestion that deep cultivating cannot show to its full advantage unless it is accompanied by increased manuring. It can be said at once that the experiment provided no support for the idea, there being no significant interaction between the manurial and the gyrotilling treatments, although there was a big response to the manures, particularly when they were drilled with the seed; no further reference will be made to the manurial comparison in this paper. The size of a main plot was approximately 1/32 acre, and in this, and in all experiments described subsequently, the sampling was at random all over the plot.

Table IB. *Girton Allotment experiment. Oats, second crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| From samples | | | | | |
| No. plants per ft. (18. iv. 35) | 15·59 | 13·80 | 0·385 | 113·0 | G > O* |
| No. panicles per ft. | 7·88 | 7·46 | 0·239 | 105·6 | Insignificant |
| Wt. grain per ft. corrected for no. of panicles | 9·20 | 8·81 | 0·271 | 104·5 | Insignificant |
| From plots | | | | | |
| Wt. grain (bush./acre) | 32·46 | 29·46 | 2·106 | 110·2 | Insignificant |
| Wt. straw (cwt./acre) | 20·89 | 17·14 | 1·188 | 121·9 | Insignificant |

Results for the crop are given in Table IB, where it will be seen that the yield was not high. The gyrotilled plots looked better throughout, and it will be observed that the difference in number of plants in April was significant; this was probably a reflexion of the fact that the gyrotilled plots were rather drier at the time of drilling. No other significance, however, was obtained, although the yield of grain and straw was appreciably higher on the gyrotilled plots. It must be remembered that there were only six replications for the comparison, so that the difference would have to be very constant over all plots for significance to be obtained; in actual fact, the gyrotilled plots outyielded the controls in five of six replications, the figures for the yield of the gyrotilled plots expressed as a percentage of those of the controls being 122, 127, 113, 75, 102, 132 for the six blocks. This extremely high variation, for which no adequate explanation can be offered, naturally resulted in a high error and hence insignificance. Similar variation arose in the case of the straw.

Broad red clover was undersown in the oat crop, and a satisfactory establishment was obtained: during the following winter, however, an attack of clover sickness (*Scelerotinia trifoliorum*) killed off many of the plants, and there was considerable growth of black grass (*Alopecurus*

*agrestis*) and of charlock (*Sinapis alba*). The infestation of charlock was not very severe but was undoubtedly worse on the gyrotilled plots. The field was cut for hay on 6 June 1936, and transverse strips were weighed on 8 June, the area of a plot being 1/65 acre. The results, expressed as hay with 15 % moisture, are shown in Table I c. In this case the gyrotilled plots gave a yield 10 % lower than that of the controls, but again the difference was quite insignificant.

Table I c. *Girton Allotment experiment. Seeds, third crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| Hay (15 % water) (cwt./acre) | 31·32 | 34·54 | 1·376 | 90·7 | Insignificant |

It was intended to abandon the experiment at this stage, but to leave the pegs in the headlands of the field to mark gyrotilled plots, in case differences should appear later. Wilhelmina wheat was drilled on the whole field in the autumn of 1936. In July 1937 the crop became lodged, and differences were apparent which coincided with the gyrotilled plots. The wheat on the gyrotilled plots was riper and suffered more from lodging. The winter and spring of that season had been very wet, and it is thought that the gyrotilling may have helped drainage and so have led to earlier growth in spring. It was therefore decided to harvest another transverse strip experimentally; the area of the plot was 1/77 acre and the results obtained are shown in Table I d.

Table I d. *Girton Allotment experiment. Wheat, fourth crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| From samples | | | | | |
| No. ears per ft. | 15·41 | 15·48 | 0·664 | 99·6 | Insignificant |
| % dry matter in grain | 84·22 | 81·83 | 0·785 | 102·9 | Insignificant |
| From plots | | | | | |
| Wt. grain (bush./acre) | 55·55 | 51·86 | 1·401 | 107·1 | Insignificant |
| Wt. straw (cwt./acre) | 50·55 | 48·34 | 1·586 | 104·6 | Insignificant |

Again the results were quite insignificant. In this respect it is evident that the six replications of this experiment were insufficient to detect anything but large differences, but when the experiment was laid down large differences in one direction or the other were expected.

*Longfallen*

This field carried a crop of wheat in 1933–4, and the gyrotilling treatment was carried out immediately after harvest on 27 and 28 August 1934. On this occasion the whole field was gyrotilled except for the control plots, over which the machine passed with its skyves raised; as the ground was very hard the tracks of the "gyrotiller" made very little impression on the control plots, although it was raining when some of the gyrotilling was done. The depth of working was estimated to be about the same as on Girton Allotment, i.e. 16–18 in. The experiment took the form of a belt, 30 yd. wide, running right across the field and consisted of eight simple randomized blocks of two plots each. Thus the full length of a plot was 30 yd.; in each experiment 15 yd. or less were actually employed, but it was considered desirable to have large plots in the first place, so that lateral drainage effects might be avoided. The width of a plot was four breadths of working of the "gyrotiller", and varied from 40 to 45 ft. Shortly after the gyrotilling the whole experimental area was ploughed, this being the first time the control plots were moved. Subsequently the field was cultivated three times, and finally drilled with beans on 24 and 25 October.

Table II A. *Longfallen experiment. Beans, first crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| From samples | | | | | |
| No. plants per yd. (11. i. 35) | 16·31 | 16·13 | 0·279 | 101·3 | Insignificant |
| No. stems per yd. (13. iv. 35) | 41·25 | 40·66 | 0·755 | 101·5 | Insignificant |
| No. stems at harvest | 18·80 | 19·53 | 0·327 | 96·3 | Insignificant |
| No. pods at harvest | 73·77 | 74·66 | 1·212 | 98·8 | Insignificant |
| From plots | | | | | |
| Wt. grain (bush./acre) | 28·17 | 29·71 | 0·777 | 94·8 | Insignificant |
| Wt. straw (cwt./acre) | 36·91 | 39·15 | 1·230 | 94·3 | Insignificant |

Table II A gives the results of this experiment and shows that the gyrotilling had no significant effect on the crop at any stage of growth. On the other hand gyrotilling appeared to benefit the condition of the soil in spring because, when horse-hoeing was carried out, the labourers noticed that on the gyrotilled plots the blades ran much cleaner, and that the soil "pushed" less than on the control plots; thus it appears that the gyrotilling had a helpful effect in draining the surface soil, but the table shows that it was completely without effect on yield.

In 1935–6 a crop of Yeoman wheat was grown on the field, drilling being carried out on 7 November. It was decided to halve plots for comparison of a rough and a fine seed bed, the former being produced by dragging a section of a rigid-framed harrow across the plot several times; this was done by means of ropes, so that the cultivation did not entail extra trampling. In view of the fact that, in some cases, significant interactions were found between the two sets of treatments, the results are given in Table II B for all combinations of treatments.

Table II B. *Longfallen experiment. Wheat, second crop after gyrotilling*

| | Gyrotilled (G) | | Non-gyrotilled (O) | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Observation* ... | Rough seed bed (RG) | Fine seed bed (FG) | Rough seed bed (NO) | Fine seed bed (FO) | S.E. of a mean | G/O % | R/F % | *Significance* |
| From samples | | | | | | | | |
| No. plants per ft. (14. i. 36) | 13·40 | 13·84 | 14·10 | 13·26 | 0·512 | 99·5 | 101·5 | Insignificant |
| No. stems per ft. (16. iv. 36) | 38·49 | 40·93 | 39·18 | 40·57 | 1·539 | 99·6 | 95·3 | Insignificant |
| No. ears per ft. | 21·00 | 22·47 | 22·47 | 21·15 | 0·880 | 99·6 | 99·6 | Insignificant |
| Wt. grain per ft. corrected for no. of ears | 61·53 | 59·44 | 59·38 | 58·98 | 1·011 | 102·2 | 102·1 | Insignificant |
| From plots | | | | | | | | |
| Average % of crop standing | 73 | 46 | 44 | 45 | 6·001 | 131·9 | 128·4 | R > F*, Interaction* |
| Wt. grain (bush./acre) | 54·52 | 57·93 | 55·96 | 54·32 | 1·046 | 102·0 | 98·4 | Interaction* |
| Wt. straw (cwt./acre) | 54·35 | 58·45 | 55·58 | 53·06 | 2·391 | 103·8 | 98·6 | Insignificant |

The crop was a good one and stood well until shortly before harvest when some lodging occurred. The percentage of each plot that was lodged was carefully estimated, and Table II B shows that the figures obtained gave statistical significance; this significance was entirely due to the much better standing on the gyrotilled plots which had the rough seed bed. It is only possible to record the finding, for no adequate explanation can be offered; although the difference shown is very large, it must be remembered that the variation in amount of lodging on the various plots was extremely high, and hence the result only obtained significance at the lowest level. The only other significant result shown is that for the interaction between the treatments in regard to weight of grain. On gyrotilled land the fine seed bed outyielded the rough, whereas the reverse was true on the ungyrotilled land. It may be that the added cultivation

was beneficial after gyrotilling in making the land firmer, whereas on the unstirred plots consolidation may already have been sufficient.

Since the gyrotilling had had no effect on yield in the first two years, it was decided to halve the original plots to test the effect of a gyrotilling at this stage; thus four types of subplots were produced: gyrotilled 1934 and 1936, gyrotilled 1934, gyrotilled 1936 and not gyrotilled on either occasion. The 1936 gyrotilling was carried out on 11 September, and it was judged that the depth of working did not exceed 1 ft. Unfortunately it was not possible to plough those plots not gyrotilled in 1936 till late October. Resistance oats were drilled between 10 and 16 November, the operation being interrupted by wet weather. The difference in time of breaking the stubble was unfortunate because the autumn was wet and the field was very rough when drilled: at that time there was a marked difference in tilth in favour of the 1936 gyrotilled plots, which had had more than a month's extra weathering. This finer tilth resulted in a fuller plant establishment, as can be seen in Table IIc; thus in this

Table IIc. *Longfallen experiment. Oats, third crop after gyrotilling*

| | Gyrotilled 1934 ($G_1$) | | Non-gyrotilled 1934 (O) | | | | | |
|---|---|---|---|---|---|---|---|---|
| Observation ... | Gyrotilled 1936 ($G_2$) | Non-gyrotilled 1936 (O) | Gyrotilled 1936 ($G_2$) | Non-gyrotilled 1936 (O) | S.E. of a mean | $G_1/O$ % | $G_2/O$ % | Significance |
| From samples | | | | | | | | |
| No. plants per ft. (13. i. 37) | 20·87 | 19·80 | 22·40 | 18·29 | 0·911 | 100·0 | 113·6 | $G_2 > O$* |
| No. stems per ft. (13. iv. 37) | 46·26 | 38·24 | 43·20 | 32·33 | 1·769 | 111·9 | 126·8 | $G_1 > O$*<br>$G_2 > O$*** |
| Estimated % ground covered by weeds (15. iv. 37) | 10·02 | 8·46 | 11·08 | 6·89 | 0·930 | 102·8 | 137·5 | $G_2 > O$** |
| No. ears per ft. | 24·41 | 20·96 | 23·64 | 19·25 | 1·063 | 105·8 | 119·5 | $G_2 > O$** |
| Wt. grain per ft. corrected for no. of ears (g.) | 22·80 | 20·45 | 24·06 | 23·77 | 0·568 | 90·4 | 106·0 | Insignificant |
| From plots | | | | | | | | |
| Wt. grain (bush./acre) | 66·48 | 61·19 | 58·54 | 56·96 | 3·011 | 110·5 | 105·8 | Insignificant |
| Wt. straw (cwt./acre) | 37·36 | 33·77 | 34·36 | 29·75 | 2·361 | 110·9 | 112·9 | Insignificant |

experiment the 1936 gyrotilling had an unfair advantage over its control. On the other hand, the finer tilth led to a much greater growth of weeds, as was brought out by estimations made in April of the percentage of the surface covered by weeds.

The only significant result for 1934 gyrotilling was in the number of stems in April, which was increased by 12 %, and this may be taken as evidence that in the very wet spring of 1937, the gyrotilling of two and a half years before was still having a helpful effect on drainage; there was in fact an increase in grain yield associated with 1934 gyrotilling of 10 %, but this failed to be significant. The 1936 gyrotilling produced highly significant results in the early stages of growth, but these faded out at harvest. From Table II c it might be gathered that the crop was too thick, but its appearance during growth never gave that impression.

### *Bunkers Field*

This experiment was designed as a simple comparison on eight randomized blocks of gyrotilling on the one hand, and of ploughing and subsoiling with horses on the other, as a preparation for sugar beet after barley. Gyrotilling was carried out in late August 1934, the plots being arranged as on Longfallen. As in that experiment, the plots were marked out as the gyrotilling proceeded and the non-gyrotilled plots were ploughed and subsoiled in early September; subsequently farmyard manure was applied and the whole area ploughed. At the end of the summer of 1934 the ground was very dry and hard and the "gyrotiller" only worked to a depth of 12–14 in.; the subsoiling extended to a depth of 8 or 9 in. There is no doubt that the "gyrotiller" brought a certain amount of subsoil to the surface: the subsoil on this field is practically pure gravel and the gyrotilled plots were conspicuous in the following year because of the gravel on the surface. Since the autumn of 1934 the whole field has been treated in the usual farm manner as regards cultivations. The sugar beet was drilled on 6 April 1935, the drill rows running down the belt of plots, i.e. across the direction of gyrotilling. The crop on the plots was lifted on 8 and 9 October 1935, the size of plot actually lifted being 1/38 acre.

Table III A. *Bunkers Field experiment. Sugar beet, first crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| No. roots per acre | 18,793 | 18,473 | 132 | 101·7 | Insignificant |
| % bolters | 3·31 | 3·19 | 0·269 | 105·7 | Insignificant |
| % dirt tare | 12·02 | 11·38 | 0·962 | 105·7 | Insignificant |
| Washed beet (tons/acre) | 14·53 | 14·63 | 3·175 | 99·3 | Insignificant |
| % sugar | 15·51 | 15·47 | 0·085 | 100·2 | Insignificant |
| Wt. sugar (tons/acre) | 2·256 | 2·265 | 0·047 | 99·6 | Insignificant |
| Wt. tops (tons/acre) | 10·64 | 10·15 | 0·392 | 104·9 | Insignificant |

The results are shown in Table III A, where it will be seen that significance was not reached in any case. It was thus very clear that, on that particular field, and in that particular season, gyrotilling was neither better nor worse than ploughing and subsoiling as the first cultivation in preparation for sugar beet.

The next crop was Spratt-Archer barley which was drilled in the normal way on 19 March, broad red clover seeds being undersown on 23 March. No developmental studies were made on the barley crop throughout its growth, and at harvest time it was impossible to cut samples for detailed study, because the clover grew very luxuriantly, in many places overtopping the barley. When the plots were cut it was necessary to comb out the clover by hand and consequently a small-sized plot (1/129 acre) was used. The results are shown in Table III B, where it will be observed that with both grain and straw there was

Table III B. *Bunkers Field experiment. Barley, second crop after gyrotilling*

| Observation ... | Gyrotilled (G) | Non-gyrotilled (O) | S.E. of a mean | G/O % | Significance |
|---|---|---|---|---|---|
| Wt. grain (bush./acre) | 37·52 | 41·45 | 0·867 | 90·5 | O > G* |
| Wt. straw (cwt./acre) | 31·18 | 34·68 | 0·883 | 89·9 | O > G* |

a decrease of approximately 10 % in yield associated with gyrotilling. A probable explanation of this effect lies in the fact that the "gyrotiller" brought an appreciable amount of pure gravel to the surface and so, as it were, diluted the already thin top soil with inert material: barley being a shallow rooted plant would be most likely to suffer from the lower concentration of plant food available.

After the barley crop was harvested the red clover grew abundantly, and it was decided to superimpose a trial of the effect of grazing with sheep in the autumn on the main plots. For this purpose each main plot was halved, and one half was grazed with sheep between mid-October and mid-November: a subplot was 1/25 acre and carried six sheep for approximately three days.

The clover was cut for hay on 4 June 1937. Sample weighings of this cut produce were made on 7 June, a distance of 10 ft. for ten swathes being used for each plot: owing to the fact that swathes in one block were mixed by the turning of the mowing machines, experimental results could only be obtained for seven blocks. A second cut was made on 12 July when seven swathes, each 12 ft. long, were weighed per subplot, all blocks being included. The results given in Table III C show no significant differences.

Table IIIc. *Bunkers Field experiment. Red clover, third crop after gyrotilling*

| Observation ... | Gyrotilled (G) Sheeped (GS) | Gyrotilled (G) Not sheeped (GO) | Non-gyrotilled (O) Sheeped (OS) | Non-gyrotilled (O) Not sheeped (OO) | S.E. of a mean | G/O % | S/O % | Significance |
|---|---|---|---|---|---|---|---|---|
| Hay | | | | | | | | |
| First cut (cwt./acre) | 45·13 | 40·26 | 45·53 | 41·84 | 2·609 | 97·8 | 110·4 | Insignificant |
| Second cut (cwt./acre) | 19·92 | 19·48 | 21·34 | 19·46 | 0·667 | 96·6 | 105·9 | Insignificant |
| Total | 65·12 | 59·91 | 67·06 | 61·16 | 3·013 | 97·5 | 109·2 | Insignificant |

*Note.* (1) The additions in this table do not quite check, because lines 1 and 3 refer to seven blocks, whereas line 2 refers to eight blocks.
(2) In all cases the yields have been adjusted to hay containing 15 % moisture.

### *Dry Field*

In view of the failure of the Bunkers Field experiment to detect any difference between gyrotilling and ploughing and subsoiling as preparation for sugar beet, it was decided, in the case of Dry Field, to include a third treatment—ploughing alone. The experiment consisted of seven randomized blocks for the three treatments included, and again took the form of a belt running across the field, the remainder of which was all gyrotilled; the width of plot was reduced from four to three "gyrotiller" breadths. Gyrotilling was carried out in this experiment at the end of August 1935 when the ground was in a very dry and hard condition. The depth of working was extremely variable, but probably averaged about 12 in. It was noted at the time that less subsoil gravel was brought to the top than on Bunkers Field. The ploughing and subsoiling could not be carried out until some two months after the gyrotilling had been completed, though the experiment did not indicate that the delay had any important effects on the results.

To gather further information on the relationship between deep cultivation and heavy manuring, each of the main plots was divided into two for a comparison of heavy and light manuring. The whole field received, in addition to a dressing of farmyard manure and 1 cwt. of nitro-chalk after the beet were singled, 2 cwt. of sulphate of ammonia, 3 cwt. of superphosphate and 1½ cwt. of muriate of potash per acre on the seed bed; the seed-bed dressing was applied to the winter furrow immediately before the working of the seed bed, and was doubled in the case of the heavy manured plots. Apart from this the whole field was treated in the normal farm manner, being sown with sugar beet in April. Throughout

the summer there was no difference between the beet receiving different cultivations, but the highly manured plots stood out distinctly all through the period of growth. The crop was an exceedingly good one.

Two separate liftings were made—on 7 October and 23 November—the upper half of the whole area being lifted on the first occasion and the lower on the second: the reason for making two liftings was that it was thought that the highly manured plots might keep growing later into the autumn than the low manured ones. It will be realized that the significance of the difference between liftings and treatments could not be determined, since the halves of the subplots were not randomized over the liftings. In point of fact the results obtained at the two liftings were closely similar and Table IV A gives the results for the two liftings combined, i.e. with the yields of the upper and lower part of each subplot added together. For this reason figures for dirt tare and sugar percentage cannot be shown, but it may be said that at neither lifting was there any significant difference between the treatments in respect of either of these two variables. Efforts were also made to determine the effects of the treatments on the shape of the roots, and estimates at the first lifting indicated that both the gyrotilling and subsoiling had given straighter and less "fangy" beet. Estimates made at the second lifting, however, failed to bring out any significant difference between any of the treatments. The subsoiling treatment definitely decreased the number of roots per acre, Table IV A showing the difference to be very highly significant. The only reasonable explanation that can be offered for this depression is that the subsoiling, having been done two months later than the gyrotilling, its plots were relatively "hollow" at the time of drilling, and that this led to a poor plant establishment. It must be added, however, that no difference was detected at the time of drilling, nor could any difference be seen during the growth of the crop. As regards yield, the subsoiled plots were significantly higher than the gyrotilled, both with weight of washed roots and in weight of sugar, but neither of these differed significantly from the control (i.e. ploughed only). There were low correlations between figures for yield and number of roots, and consequently correction for the latter, although widening the differences, destroyed the significances. As regards level of manuring many significant differences emerged, but for the purpose of this paper it is only necessary to state that there was no case of a significant interaction between level of manuring and cultivation treatment.

In the following year Spratt-Archer barley was drilled on 2 April, the experimental area being worked with the rest of the field. No

Table IVA. *Dry Field experiment. Sugar beet, first crop after gyrotilling*

| Observation ... | Cultivation treatments: Gyro-tilled (G) | Sub-soiled (S) | Control (C) | S.E. of a mean | G/C % | S/C % | Significance | Manurial treatments: High level (H) | Low level (L) | S.E. of a mean | H/L % | Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. roots per acre | 29,141 | 27,200 | 29,338 | 305 | 99·3 | 92·7 | C, G > S*** | 28,480 | 28,639 | 237 | 99·4 | Insignificant |
| No. bolters per acre | 394 | 372 | 431 | 54 | 91·4 | 86·3 | Insignificant | 479 | 319 | 52 | 150·2 | H > L* |
| Wt. tops (tons/acre) | 9·77 | 9·83 | 9·81 | 0·432 | 99·6 | 100·2 | Insignificant | 10·95 | 8·66 | 0·140 | 126·4 | H > L*** |
| Wt. washed roots (tons/acre) | 17·94 | 18·70 | 18·34 | 0·228 | 97·8 | 102·0 | S > G* | 18·83 | 17·82 | 0·208 | 105·7 | H > L** |
| Wt. sugar (tons/acre) | 3·025 | 3·174 | 3·115 | 0·040 | 97·1 | 101·9 | S > G* | 3·174 | 3·035 | 0·033 | 104·6 | H > L** |
| Wt. tops corrected for no. roots | 9·80 | 9·81 | 9·79 | 0·294 | 100·1 | 100·2 | Insignificant | 10·95 | 8·66 | 0·455 | 126·4 | H > L*** |
| Wt. washed roots corrected for no. roots | 17·86 | 18·91 | 18·22 | 0·382 | 98·0 | 103·8 | Insignificant | 18·84 | 17·79 | 0·431 | 105·9 | H > L** |
| Wt. sugar corrected for no. roots | 3·017 | 3·192 | 3·104 | 0·047 | 97·2 | 102·8 | Insignificant | 3·175 | 3·034 | 0·034 | 104·6 | H > L* |

Table IVB. *Dry Field experiment. Barley, second crop after gyrotilling*

| Observation ... | Cultivation treatments: Gyro-tilled (G) | Sub-soiled (S) | Control (C) | S.E. of a mean | G/C % | S/C % | Significance | Manurial treatments: High level (H) | Low level (L) | S.E. of a mean | H/L % | Significance |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| From samples | | | | | | | | | | | | |
| No. ears per ft. | 14·65 | 14·87 | 13·92 | 0·339 | 105·2 | 106·8 | Insignificant | 14·72 | 14·24 | 0·246 | 103·4 | Insignificant |
| Wt. grain corrected for no. of ears | 10·15 | 10·29 | 10·86 | 0·392 | 93·4 | 94·7 | Insignificant | 10·28 | 10·59 | 0·207 | 97·0 | Insignificant |
| From plots | | | | | | | | | | | | |
| Wt. grain (bush./acre) | 30·10 | 30·30 | 31·16 | 1·006 | 96·6 | 97·2 | Insignificant | 31·00 | 30·04 | 0·846 | 103·2 | Insignificant |
| Wt. straw (cwt./acre) | 20·18 | 20·18 | 20·89 | 0·602 | 96·7 | 96·7 | Insignificant | 20·20 | 20·64 | 0·561 | 97·8 | Insignificant |
| Wt. grain corrected for wt. of straw | 30·41 | 30·60 | 30·56 | 0·741 | 99·5 | 100·1 | Insignificant | 31·30 | 29·75 | 0·443 | 105·2 | H > L* particularly on G* |

developmental studies were undertaken during the growth of the crop, which was harvested on 19 August. It was decided to continue with the original plots unchanged, with the object of seeing whether the residues from the high manuring of the sugar beet would be detected in the yields of the barley crop. The size of plot taken at harvest was 1/145 acre. The results are shown in Table IV B.

The only significant result obtained was in regard to weight of grain corrected for weight of straw, in which case high manuring was 5 % higher than low manuring, and also there was a significant interaction between the two sets of treatments; the interaction was a high response (15 %) to high manuring on the gyrotilled, but low response on the ploughed and subsoiled, plots. This is the only occasion in which evidence was obtained in support of the belief that deep cultivation should be accompanied by heavy manuring, but significance only arose in grain/straw ratio, the same effect being indicated, without significance, in the case of yield of grain.

## Discussion

The only general conclusion that can be drawn is that in these experiments gyrotilling has had very little effect on crop yield; in twelve experiments on the four fields the difference between gyrotilling and its controls, in actual yield, was insignificant in eleven cases. Very little weight can be attached to insignificant differences, but the actual increases or decreases obtained in final yield are collected in Table V, and it will be observed that on the heavy land the difference was in favour of gyrotilling in five of seven cases. Column 5 of Table V shows how large a percentage difference was necessary in each case for significance ($P < 0{\cdot}05$); most of these latter figures can be regarded as satisfactory, the one bad case being that for oats on Girton Allotment, where five blocks favoured the gyrotiller whilst the remaining one gave a large difference against it. The values shown in the last column must be read with the greatest reserve, but in so far as they can be trusted it appears that over a period of four years gyrotilling just paid for itself on Girton Allotment; that over three years about half the cost was repaid on Longfallen (neglecting the second gyrotilling), whilst on both light land fields its effect was uniformly harmful. General experience indicates that gyrotilling does not reduce subsequent cultivations, and consequently the operation should be regarded as involving extra cost, to the full amount paid to the contractor.

Table V. *Summary of differences in yield*

| Field | Soil type | Year after gyro-tilling | Crop | Minimum % difference to give significance ($P < 0.05$) | % increases in yield over normal cultivation | Value per acre of difference in yield s. d. |
|---|---|---|---|---|---|---|
| Girton Allotment | Gault clay | 1st | Wheat | 6·7 | 3·1 | 9 10 |
| | | 2nd | Oats | 21·9 | 10·2 | 9 5 |
| | | 3rd | Seeds Hay | 15·2 | −9·3 | −9 8 |
| | | 4th | Wheat | 9·5 | 7·1 | 20 10 |
| Longfallen | Gault clay | 1st | Beans | 9·0 | −5·2 | −7 8 |
| | | 2nd | Wheat | 2·4 | 2·0 | 6 1 |
| | | 3rd | Oats | 13·0 | 10·5 | 19 0 |
| Bunkers Field | Gravel loam | 1st | Sugar Beet | 6·9 | −0·4 | −2 1 |
| | | 2nd | Barley | 7·3 | −9·5 | −19 7 |
| | | 3rd | Seeds Hay | 14·1 | −2·5 | −9 7 |
| Dry Field | Gravel loam | 1st | Sugar Beet | 3·8 | −4·7 | −34 4 |
| | | 2nd | Barley | 10·1 | −0·7 | −1 0 |

For the final column the following values have been taken:

| | | | |
|---|---|---|---|
| Wheat | 45*s.* per qr. | Hay | 60*s.* per ton. |
| Oats | 25*s.* per qr. | Beans | 40*s.* per qr. |
| Barley | 40*s.* per qr. | Sugar Beet | 11*s.* 6*d.* per cwt. sugar. |

It is, of course, impossible to measure the value of a cultivation treatment entirely in terms of yield, although that must always be the main consideration. On heavy land gyrotilling does appear to help drainage, and several observations have been made which show that it may facilitate subsequent tillages. Against this must be set the possibility that it may favour the absorption of rain and lead to something resembling a morass, and also to the possibility in dry weather of extra cultivation being necessary in working down the seed bed for the first crop after gyrotilling. The effect on weeds is also an important consideration, but in these experiments no important differences in this respect were seen between the gyrotilled plots and their controls. It might be urged in favour of the "gyrotiller" that it is a convenience to the farmer at rush periods, since it requires no attendance and leaves no tracks behind it.

## Summary

1. In two experiments on heavy land (one continued for four, and the other for three years) gyrotilling has been compared with normal cultivations, and no significant increase in yield has been obtained, although

in five out of seven experimental crops the actual difference was very slightly in favour of gyrotilling.

2. In two experiments on light land (one continued for three, and one for two years) gyrotilling has been compared with normal cultivations (ploughing and subsoiling, and also, in one case, ploughing alone), and in all five experimental crops there was a slight depression in yield, which was only significant in one case, connected with gyrotilling.

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# THE APPROXIMATE RECOVERY OF INFORMATION FROM REPLICATED FIELD EXPERIMENTS WITH LARGE BLOCKS

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1. In recent years the tendency for experiments with large blocks (that is, blocks with a large number of plots) to be correspondingly variable has been met in more than one way according to the nature of the experiment. For experiments of factorial type, it is often convenient to reduce the block size by confounding; for other experiments, e.g. variety trials, it has been suggested by Yates that a quasi-factorial pattern can be superimposed on the treatments or varieties, and analogous methods of reducing the block size hence made possible (see, for example, Yates, 1937). Provided an experiment is well designed for its purpose, such valid modifications from straightforward randomized block layouts should result in increased efficiency with very little extra computation in the statistical analysis.

In one of his publications on the technique and results of field experiments, Papadakis has, however, put forward the proposal that an explicit use of the correlation between neighbouring plots should be made, and the usual method of blocks be replaced by a covariance method (Papadakis, 1937).[1] Papadakis' presentation of his method is stimulating, but somewhat uncritical; and it is the purpose of this paper to examine more fully the validity and value of his proposals.

The method is, briefly, to correct the yield of each plot for the average effect over the replications of the treatment actually applied to the plot, and the mean of the corrected yields of the two (say) plots contiguous to it is then used as a concomitant observation with which covariance may be used. This seems to be an extension of previous attempts by various writers to allow for the yields of neighbouring plots without recourse either to the indirect method of blocks (and its various modifications) or to the insertion of actual controls; and is rather an ingenious attempt to use in an efficient manner *all* the plots as controls.

[1] I am indebted to Mr G. E. Blackman for first drawing my attention to Papadakis' paper.

Besides the reduction in the error, which Papadakis points out is likely to be most marked when the plots are long and narrow or the total number of plots increased, Papadakis claims that

"*La méthode présente aussi les avantages suivants:*

(1) *Elle ne demande pas une disposition ad hoc des parcelles.*

(2) *Elle peut être employée avec une disposition systématique par laquelle l'erreur est diminuée.*

(3) *L'erreur n'est pas augmentée avec l'augmentation de l'étendue totale du champ d'expériences. On peut par conséquent étudier sur le même champ un grand nombre de variétés et profiter de la variété de conditions écologiques, que présente un grand champ. Cette variété de conditions rend un champ hétérogène préférable à un champ homogène.*

(4) *Le nombre des répétitions peut être différent pour les différentes variétés, d'après leur importance et leur semence disponible.*"

My own opinion on the extent to which the use of the method seems justified will become apparent from the results and conclusions of this paper. First of all, it will be convenient to familiarize the reader further with the proposed method by illustrating its application to two experiments with large fully randomized blocks. At this stage comment is mainly deferred, though it will appear in due course that these experiments were of a type to which an application of the method appears suitable.

2. Of a series of large-scale experiments in Egypt on cotton during the years 1934–6,[1] two in 1936, although of factorial type, were completely randomized within blocks, which each contained seventy-two plots. This course was adopted partly because of the apparent uniformity of the sites on which these two particular experiments were put down, partly on the indication from previous years that for experiments laid out on the main plot, subplot, principle, the information that would have been lost on the subplot treatments by complete randomization within blocks was usually not too great. The percentage standard errors per plot for these two experiments (designated by their location in Egypt) were 8·9 for Tukh and 12·0 for Mahallet Roh. These errors were low enough to make the treatment effects at these centres fairly definite: the extra investigation given below was undertaken later for the purpose of the present theoretical enquiry.[2]

[1] For a review of the results of these experiments, see Crowther *et al.* (1937); for technique, Crowther & Bartlett (1938).

[2] Dr F. Crowther kindly let me use the yield figures for this purpose.

Both experiments had three replications, plots and blocks being compact in shape, the latter containing $8 \times 9$ plots, and the entire sites $24 \times 9$ plots. The factorial systems of treatments were:

Mahallet Roh: Nitrogen (4) × Spacing (3) × Varieties (3) × Phosphate (2).

Tukh: Nitrogen (4) × Sand Sowing (2) × Method of application of nitrogen (3) × Date of application (3).

(The figures in parentheses denote number of levels or treatments corresponding to each factor. Incidentally, since any fertilizer factor always contained a zero level, some of the treatment combinations in the Tukh experiment were "dummy".)

In each experiment the yields of all plots were corrected for the treatment effect; that is, the quantities

$$y' = y - \bar{y}_t + \bar{y}$$

calculated, where $y$ represents the yield of any plot, $\bar{y}_t$ the average yield of the three plots (including the given one) with the same treatment, $\bar{y}$ the average yield of all the plots. Since the plots were compact in shape, the mean value of the $y'$'s for the *four* plots each contiguous along one side to the given plot was next calculated for each plot. Block classification was here ignored, so that this was possible except for edge plots, for which the mean of the three contiguous plots was taken; for each of the four extreme corner plots in the site, the mean of the two contiguous plots was taken. The mean of these $y'$'s is denoted by $x$, and the remaining analysis is simply the use of covariance between $y$ and $x$ to adjust the straightforward analysis of variance of $y$. There is no need here to reproduce the whole of these analyses, nor to give the full final analysis of variance, but the items at each centre before and after adjustment are given in Table I for Total Treatments, Blocks and Error. The approximate method of correcting the analysis of variance items was used for convenience in preserving the additive character of

Table I. *Analysis of variance (sums of squares before and after adjustment)*

| | | Mahallet Roh | | Tukh | |
|---|---|---|---|---|---|
| | D.F. | $y^2$ | $y_x^2$ | $y^2$ | $y_x^2$ |
| Total treatments | 71 | 286·9459 | 271·9719 | 230·0407 | 199·2953 |
| Blocks | 2 | 50·7032 | 4·5759 | 4·4707 | 0·0862 |
| Error | 142* | 62·6249 | 32·0671 | 62·9912 | 38·1587 |
| Grand total | 215* | 400·2740 | 308·6149 | 297·5026 | 237·5402 |

* Two degrees of freedom less for adjusted analysis (see § 3). Also the error term for Tukh does not include the comparisons between "dummy" treatments.

the table, but of course the significance of any item in the full set of treatment effects could be tested somewhat more exactly if required (see, for example, Fisher, 1934, p. 266).

The percentage standard error for Mahallet Roh was reduced from 12·0 to 8·6 %. This was not altogether surprising, for while a great deal of the variation in the site was between blocks, the fact that such block differences existed suggested the existence of further fertility gradients within the blocks. The reduction in the error at Tukh from 8·9 to 7·0 % was a little more unexpected, owing to the greater uniformity of this site.

Treatment differences (these individual items are not shown here) at Mahallet Roh were little affected by the adjustment. The small average phosphate effect became significant, the positive nitrogen × phosphate interaction, which was formerly significant, was afterwards insignificant for the average of its three degrees of freedom, but the dominant term, phosphate × nitrogen regression, was still definitely significant. One apparent anomaly was a significant nitrogen × spacing × phosphate interaction after adjustment. This could not readily be interpreted in terms of real effects, owing to the apparent absence of spacing effects in this experiment, and it is possible that it was a chance inflation due to a rather large $x^2$ item corresponding to this treatment effect. This $x^2$ item, together with two others, appeared themselves when compared with their own error in the neighbourhood of $P = 0{\cdot}05$ significance, but this may quite well have been a chance effect; at least a test of the homogeneity of these $x^2$ items, as an internal check on the validity of the method, gave the following satisfactory result (for the method of making this test, see Bartlett, 1937, p. 158).

Table II. $\chi^2$ *test of variances* ($x^2$ *analysis, Mahallet Roh*)

| | D.F. | $\chi^2$ (crude value) |
|---|---|---|
| Among treatment items in analysis of variance table | 14* | 17·09 |
| Treatments v. error | 1 | 0·62 |
| Total | 15 | 17·71 |

* The 15 items consist of main effects, 4; first order interactions, 6; second order, 4; third order, 1.

At Tukh no treatment differences were affected, nor did any $x^2$ items happen to approach significance.

The adjustment of the treatment means in any summary tables would follow of course the usual covariance procedure. For such adjusted

means it would be quite sufficient, especially as the number of degrees *of freedom for the error turn is large*, to allot a common standard error *corresponding to the corrected error variance.*

The reduction in the block differences is of interest. The method of adjustment will usually have this effect; but conversely, it is important to note that well-arranged blocks may succeed in eliminating automatically much of the variation that would otherwise remain until eliminated by this method.[1]

3. The above examples have been inserted as an introduction to a further discussion of the value of Papadakis' suggestions. The points to be considered, in this order, are the theoretical validity (or approximate validity) of the proposed method of adjustment, and its apparent practical value, the latter depending on various considerations, such as validity, efficiency and degree of simplicity.

It is fairly evident, owing to the double use of each plot yield for both dependent and independent variate in the analysis of variance, that under no conditions can the method lead *exactly* to the usual analysis of variance and covariance theory. But it is equally evident that a close and homogeneous correlation between neighbouring plots can be largely eliminated by an application of the method, and it still remains to determine under what conditions the theoretical weaknesses might be sufficiently negligible to make the method permissible.

This problem will be dealt with in stages. It will first be supposed that no *real* treatment or positional effects are operating. The reduction in the error sum of squares is then due to the use of a regression coefficient obtained between a series of independent plot yields and certain linear functions of *other* plot yields in the *same* series. In such circumstances it is easily seen that the mean covariance is never greater than zero, the elimination of any general or block means converting the exactly zero mean to some artificial negative value. This negative correlation will be of the order of magnitude, $1/(p-1)$, where $p$ is the smallest number of plots represented in the means so eliminated.

The variance of the correlation requires a little more consideration. For a series of $n$ independent deviations, the variance of the serial correlation between successive pairs is about $1/n$ when $n$ is large (Bartlett,

[1] An attempt to apply the method to an extremely variable experiment on vines in France met with little success. The block differences, which were fairly marked, were insignificant after adjustment; but the extra variation within blocks eliminated by the adjustment, while it appeared significant, did not materially reduce further the very large error.

1935, p. 537). This result was obtained by finding first the variance of the covariance, and noting that if the mean covariance is zero, fluctuations in any divisor are of minor importance. It follows in the same way that the corresponding analysis-of-variance regression term will have approximately the correct mean square,

$$E\left\{\frac{(\Sigma y_r y_{r-1})^2}{\Sigma y_{r-1}^2}\right\}=\sigma_y^2.$$

If, however, we correlate $y_r$, not with $y_{r-1}$, but with, say, $x_r=y_{r-1}+y_{r+1}$, the sum of products $\Sigma x_r y_r$ can be written approximately $2\Sigma y_r y_{r-1}$, the effect at the ends of the series being neglected. Hence when we square this term, and divide by $\Sigma x_r^2$, or by $2n\sigma_y^2$ approximately, since the variance of $x_r$ is $2\sigma_y^2$ if the $y_r$ are really uncorrelated, we obtain the result

$$E\left\{\frac{(\Sigma y_r x_r)^2}{\Sigma x_1^2}\right\}=2\sigma_y^2.$$

From general considerations this result would not be affected if the regression term were obtained from modified deviations corresponding to fewer degrees of freedom; but it *is* affected in the present problem by the initial adjustment necessary to correct for treatment effects.

The effect of this adjustment is briefly sketched in the following algebraic discussion. Let the number of replications be $\lambda$; the number of treatments, $t$. The yields adjusted for treatments may be denoted by

$$w=\frac{\lambda-1}{\lambda}\,y-\frac{1}{\lambda}\,y'-\frac{1}{\lambda}\,y''-\dots$$

for plots in one block, $y'$, $y''$, etc. being the yields for the plots in the other blocks with the same treatment as $y$. Instead of $y_{r-1}+y_{r+1}$, we have to consider $w_{r-1}+w_{r+1}$. The total sum of products (ignoring the elimination of the general mean, or possibly of block means) is then

$$\Sigma y_r\,(w_{r-1}+w_{r+1})=\Sigma y_r\left(\frac{\lambda-1}{\lambda}\,y_{r-1}-\frac{1}{\lambda}\,y_{r-1}'-\frac{1}{\lambda}\,y_{r-1}''\dots\right.$$
$$\left.+\frac{\lambda-1}{\lambda}\,y_{r+1}-\frac{1}{\lambda}\,y_{r+1}'-\frac{1}{\lambda}\,y_{r+1}''\dots\right).$$

The point to notice is that while $\Sigma y_r\,(y_{r-1}+y_{r+1})=2\Sigma y_r y_{r-1}$ approximately, as before, $y_{r-1}'$ and $y_{r+1}'$ merely refer to plots associated with the neighbouring yields $y_{r-1}$ and $y_{r+1}$ in the first block, and the suffices $r-1$ and $r+1$ give no indication of the positions of these corresponding plots in the second block. There is in general, especially when $t$ is much larger than $\lambda$, little or no coalescence of terms in the above sum of

products, apart from those with the coefficient $\frac{\lambda-1}{\lambda}$. It follows that while the mean value,

$$E\Sigma\,(w_{r-1}+w_{r+1})^2=2\left\{\left(\frac{\lambda-1}{\lambda}\right)^2+\frac{1}{\lambda^2}+\frac{1}{\lambda^2}+\ldots\right\}\lambda t\sigma_y^2$$
$$=2\left\{\left(\frac{\lambda-1}{\lambda}\right)^2+\frac{\lambda-1}{\lambda^2}\right\}\lambda t\sigma_y^2$$
$$=2\,(\lambda-1)\,t\sigma_y^2,$$

the mean value of $E\,\{\Sigma y_r\,(w_{r-1}+w_{r+1})\}^2$ does not depend simply on a corresponding factor $4\,(\lambda-1)\,t$, but is approximately

$$\left\{4\left(\frac{\lambda-1}{\lambda}\right)^2+2\,\frac{\lambda-1}{\lambda^2}\right\}t\sigma_y^4,$$

giving for the ratio,

$$\left\{1+\frac{\lambda-1}{\lambda}\right\}\sigma_y^2.$$

The use of four contiguous plots instead of two in the case of compact plots and blocks is easily shown to have no material effect on the above result.

We have, however, still to consider the effect of using the "error terms" in the analysis of variance to calculate the regression of $y$ on $w$, rather than using the "total" sums of squares and products. This modification does now affect our result. The reduction in the degrees of freedom gives for the mean value of the error run of squares of the "independent variate",

$$2\,(\lambda-1)\,t\sigma_y^2\times\frac{(\lambda-1)\,t}{\lambda t}=2\,\frac{(\lambda-1)^2}{\lambda}\,t\sigma_y^2.$$

For the sum of products we need only consider the "dependent variate" $y$ corrected for treatments (cf. the familiar formula $\Sigma\,(x-\bar{x})\,(y-\bar{y})=\Sigma x\,(y-\bar{y})$), giving the product term $\Sigma w_r\,(w_{r-1}+w_{r+1})$. As before, only the terms $y_r y_{r-1}$ coalesce when we substitute for the $w$'s, and the mean square of the product sum gives

$$\left[4\left(\frac{\lambda-1}{\lambda}\right)^4+2\left\{2\,\frac{(\lambda-1)^2}{\lambda^4}\,(\lambda-1)+\frac{(\lambda-1)^2}{\lambda^4}\right\}\right]t\sigma_y^4$$

and the regression mean square reduces to

$$\left\{1+\left(\frac{\lambda-1}{\lambda}\right)^2\right\}\sigma_y^2.$$

It thus follows in general that the average contribution of the regression term occurring in the analysis of variance will lie between once and twice the true error variance (provided the artificial negative

correlation arising from the elimination of general or block means can be neglected). It would therefore seem advisable to subtract *two* degrees of freedom from the corrected error term; but, in any case, if the bias in the estimate of error from the remaining degrees of freedom is to be small, the number of these degrees of freedom must be large (say at least over 30). Owing to the difference in the expectation for the regression term according to whether it is calculated from the total or the error terms, it also follows that if the treatments sum of squares is obtained by subtraction the treatments mean square will have a bias of the same order. This last effect is of correspondingly less importance for isolated treatment effects with fewer degrees of freedom.

The algebraic results obtained above are only a part of the required theory, but serve to stress that this theory must necessarily be of the "large-sample" type, and with this conditiou the theory of analysis of variance reduces largely to the question of mean values and unbiassed estimates of their errors, so that concentration on these points seems justifiable.

If we now suppose real positional effects introduced, there is the possibility of eliminating these as far as possible by blocks, by Papadakis' use of covariance, or by both (as in the examples in this paper). The positive correlation that would indicate that the covariance adjustment would be worth while is liable to be reduced slightly by the artificial negative correlation caused by blocks, if the third procedure were adopted, but this failing will be unimportant when the number of plots per block is reasonably large. Since it appears inadvisable to use the procedure in other cases, the possibly spurious nature of a correction due to a *negative* correlation need not be stressed.

When a real correlation exists the regression mean square ordinarily consists of the real effect, plus the chance component for which the usual allowance of one degree of freedom is made. Here, however, the chance component itself seems on examination to be affected to some extent by the amount of correlation present, its expectation being pushed down somewhat nearer once the true corrected error variance, although still in excess.

If finally we consider the effect of real treatment differences, no further theoretical difficulty arises unless *real* treatments × replications interactions exist. But if they do, besides the extra error introduced in the correction for treatment effects, it is not altogether obvious whether the plot yields adjusted for average treatment effects, and the correlation obtained from them, have other limitations besides those already men-

tioned. The usual process of randomizing the plots assigned to particular treatments would appear, however, to make such possible extra limitations quite negligible, as, apart from the slight restriction that no treatment in a block can occur again in an adjacent plot, it is a matter of chance which treatments are affecting the yields used to correct any given plot yield. Such conclusions are not contradicted by the results of the two earlier analyses, which, in view of the numbers of plots involved, might be expected to be fairly sensitive to such possible effects.

The extra information recovered is not dependent simply on the average correlation within randomized groups, since the adjustment for treatments implies that the average yield $x$ has an additional and uncorrelated component depending on the number of replications and the *uncorrected* error variance. This has the effect of somewhat lowering the efficiency, but the final gain actually made in any experiment may still be considerable if there is much correlation between plots in the same block, as we have already seen.

Finally, there is the question of simplicity and the work involved in the statistical analysis required in this method. The calculations are rather laborious, and would compare unfavourably with the simple modifications necessary in the analysis of well-designed experiments in which confounding (or its analogues) has been introduced.

It will in fact have become evident from the examples and discussion here that the view certainly would not be upheld that the application of the method should in any sense become a matter of routine. Its theoretical limitations necessarily imply that it should be used with considerable caution, but while only an approximate method it may perhaps be classed with other initially unplanned applications of covariance as a potential recoverer of further information in suitable instances (the question of suitability is of course to be decided from the general character of the data, not from any apparent justification such as a reduced error variance). It will hardly, as Papadakis suggests, affect the design of experiments of the randomized block type. But since it is likely to be both most valid and most powerful when the number of plots per block is large, it might be remembered, if a straightforward block layout has been used for an experiment in which no simple or convenient method of reducing the block size existed, that if the blocks proved variable, the apparently lost information might be approximately recoverable by further analysis (cf. Crowther & Bartlett, 1938, footnote, p. 64). Large-scale completely randomized layouts are of course comparatively rare. There is also the possibility in the design of such experi-

ments of inserting appropriately placed controls, with which covariance may be used with *exact* validity; (see Yates, 1936, p. 428) but before we can judge the extent to which this course would prove sufficiently effective, further investigation on the efficiency of this latter method seems needed.

## SUMMARY

The method suggested by Papadakis of using covariance with the yields of neighbouring plots to reduce the error of replicated field experiments is illustrated on two large-scale cotton experiments. In a discussion on the validity and value of the method, it is concluded that for such experiments, where the number of plots per block is large, the method should be approximately valid and sometimes useful.

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# THE VITAMIN A CONTENT OF THE COLOSTRUM OF DAIRY COWS

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(With Two Text-figures)

The data detailed in the present paper were obtained in the course of an investigation designed to ascertain what role, if any, was played by vitamin A in the prevention of white scour in calves. This work is being continued for another season, when it is hoped to publish the results and conclusions, but it was considered that the values obtained for the vitamin A content of the colostrum of one hundred cows were of sufficient interest to be published at this stage, in the light of the conclusions and values published recently by other workers.

Daun (1933) examined the colostrum of fourteen cows of a dairy herd of shorthorns, and found that the vitamin A content of the colostrum was as much as ten to one hundred times as high as that of later milk. He concluded from his observations that the colostrum of heifers is generally richer in vitamin A than the colostrum of cows. Bauman *et al.* (1934), using Holstein, Guernsey and Ayrshire cows, found that the vitamin A content of the butterfat varied according to the breed of cows. Gillam *et al.* (1936), using four groups of two cows (Shorthorn, Ayrshire, Friesian, Guernsey), also found that the vitamin A content of the milk fat varied according to breed, but that there was also a large variation between cows of the same breed. The conclusions arrived at by these workers were based on values obtained from only a very few experimental animals, and consequently cannot be accepted *in toto* except after corroboration by an experiment carried out on a much larger scale.

In the present investigation samples were collected from one hundred cows belonging to fourteen farms in Ayrshire, Perthshire and Kirkcudbrightshire. With few exceptions the cows were of the Ayrshire breed, and seventy belonged to attested herds. The samples were collected in the months of February, March, April and May, while the animals were being stall fed. The rations used on the different farms were of a very

similar type, being composed of turnips or mangolds, hay and a little straw for maintenance, and a scientifically balanced dairy mixture for production purposes. A few farmers mixed their own production rations, but these all conformed to the usual type which necessitates 3½ lb. to the gallon of milk, although the constituents might differ very slightly. During the period between lactations, the production ration was usually fed but on a much lower scale.

### COLLECTION OF SAMPLES

Seven samples were collected from each cow. The first was taken within a few hours of calving, the second at the following morning's milking, and the others at successive daily milkings. The sample was received at the laboratory the day after it was collected, and the vitamin A content immediately estimated. This ensured that each sample was analysed the day following collection, but owing to postal delays those samples collected at week-ends were a day late in being analysed.

### TECHNIQUE

Vitamin A was assayed by the antimony trichloride method after the method of Davies (1933). A Pulfrich photometer was used instead of a Lovibond tintometer to read the blue units. The Pulfrich photometer was calibrated against the Lovibond tintometer by means of blue-coloured solutions, and a graph showing blue units against extinction coefficients prepared. Thereafter, by reading the extinction coefficients of the coloured solutions after the usual antimony trichloride techniqua, it was possible to obtain the blue-unit value of the colostrum directly from the graph. The ease in reading extinction coefficients on the photometer compared with the laborious matching of colours in the tintometer adds greatly to the accuracy of the antimony trichloride method, where speed in reading depth of colour is essential since the colour fades so very quickly. The total carotenoid content was measured by matching the yellow colour of the chloroform solution in the Lovibond tintometer. Moore's blue units and the yellow units were converted to international units by using the factors 1 B.U. $\equiv$ 0·6 I.U. and 0·6$\gamma$ carotene $\equiv$ 1·2 Y.U. Before converting the blue units to international units an allowance of one-tenth of the total yellow units was deducted. Thus the formula used was as follows:

$$(\text{Total blue units} - \tfrac{1}{10}\ \text{total yellow units}) \times 0{\cdot}6 + \text{total yellow units} \div 1{\cdot}2 = \text{total international units of vitamin A activity.}$$

Unfortunately, though the experiment started on 17 February, only blue units were estimated till 2 March, and this must be taken into account in examining the international units of the samples obtained between these dates. After the third or fourth daily sample when milk proper was starting to be secreted, the amount of carotene in the samples was too small to be estimated. This will be discussed later in the paper.

The results are recorded in Table I and are arranged in order of magnitude of the total blue units of the first samples.

It is at once apparent that the vitamin A content of the colostrum falls very quickly from the day of calving. By the third or fourth day the amount of vitamin A is very similar to the amount in milk, and is from a tenth to a twentieth of that of the sample taken within a few hours of parturition. Thus, if the young calf is to receive the maximum amount of vitamin A from its mother, it is essential that it should be allowed to obtain the colostrum as soon after calving as possible. Especially is this the case during stall feeding when the vitamin A content of the later milk would be at its minimum owing to the low carotene content of the foodstuffs constituting the cow's ration.

The values of the first colostrum samples of the hundred cows ranged from 1181 to 35 I.U. per 100 ml. This large variation takes place despite the diet of the cows being of the same type and character. If the results are examined farm by farm, it will be seen that, even on the same farm, where the diet was constant throughout the course of the experiment, the vitamin A content of the colostrum varies widely from cow to cow, ranging from 921 to 85 I.U. per 100 ml. colostrum in the case of farm A and from 920 to 111 I.U. in the case of farm D. From the point of view of farm management, this finding is of some importance, since it appears that it is impossible to gauge within wide limits the amount of vitamin A which one calf is obtaining, compared with that obtained by another calf on the same farm.

The distribution of frequency is very symmetrical, as is seen in Fig. 1, which depicts the logarithmic curve of the international units of the first day samples. For random observations on biological material, the symmetry of the distribution is remarkable.

In the present experiment most of the cows were of the same breed. Therefore, the influence of breed on the vitamin A content of the colostrum may be disregarded. Moreover, when the range of values for the same breed is so great, it is difficult to visualize that breed could significantly affect the vitamin A content of the colostrum. Therefore, it would appear desirable that the experiments of Gillam *et al.* and

Baumann *et al.*, already mentioned, be repeated, using greater numbers of experimental animals before it is accepted that the vitamin A content of butter-fat can show any significant variation due to difference in breed.

Date of parturition had no effect on the amount of vitamin A in the colostrum since, in many cases, cows belonging to the same herd

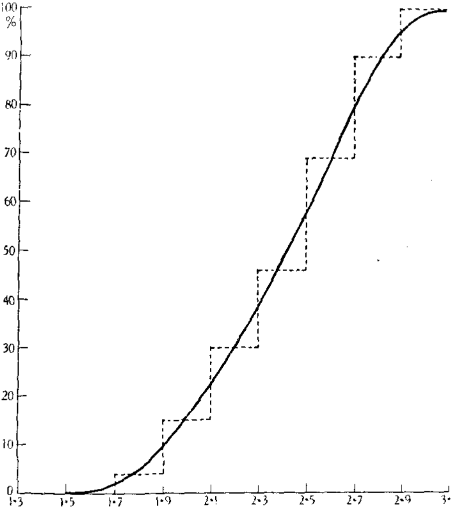


Fig. 1.

showed large differences though calving within a few days of each other—examples of this being nos. 3 and 60 and nos. 4 and 79. It must be remembered, however, that in the present experiment all the cows were stall fed. A different result might have been obtained if the experiment had included cows which had calved at grass in the early summer or even cows which had been "outwintered".

Dann (1933) stated that the colostrum of heifers had always a higher

**Table I.** *Table showing farm, age of cow, date of parturition, length of non-lactating period and the total blue units, total yellow units and international units of vitamin A of the colostrum of 100 cows for the first 7 days after parturition*

| Cow no. | Farm | Age of cow Years | Date of parturition 1937 | Total blue units (Moore's) per 100 ml. colostrum Samples | | | | | | | Total yellow units per 100 ml. colostrum Samples | | | | | | | International units of vitamin A per 100 ml. colostrum $\left\{(\text{T.B.U.} - \frac{1}{10}\text{T.Y.U.}) \times 0{\cdot}6 + \frac{\text{T.Y.U.}}{1{\cdot}2}\right\}$ Samples | | | | | | | Length of non-lactating period Weeks |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| 1 | C | 5 | 28 Mar. | 1280 | 455 | 443 | 140 | 108 | 185 | 122 | 420 | 80 | 54 | — | — | — | — | 1093 | 335 | 308 | 84 | 65 | 111 | 73 | 17 |
| 2 | N | 3 | 20 Mar. | 1035 | 712 | 266 | 74 | 77 | 47 | 139 | 95 | 64 | — | — | — | — | — | 695 | 177 | 160 | 44 | 46 | 28 | 83 | Heifer |
| 3 | J | 5 | 30 Mar. | 999 | 662 | 353 | 238 | 243 | 112 | 92 | 550 | 240 | — | 48 | 34 | — | — | 1018 | 583 | 212 | 180 | 172 | 67 | 55 | 14 |
| 4 | A | 7 | 22 Mar. | 994 | 392 | 139 | 166 | 108 | 52 | 97 | 420 | 222 | — | — | — | — | — | 924 | 407 | 83 | 100 | 65 | 31 | 58 | 17 |
| 5 | B | 9 | 23 Feb. | 940 | 387 | — | 160 | 83 | — | — | — | — | — | — | — | — | — | 564 | 232 | — | 96 | 50 | — | — | 12 |
| 6 | L | 4 | 2 Apr. | 940 | 216 | 112 | 40 | 40 | 54 | 54 | 560 | 98 | — | — | — | — | — | 997 | 206 | 67 | 21 | 24 | 32 | 32 | Heifer |
| 7 | K | 8 | 20 Mar. | 936 | 504 | 396 | 209 | 126 | 106 | 153 | 800 | 160 | 400 | — | — | — | — | 1181 | 126 | 547 | 125 | 97 | 64 | 109 | 12 |
| 8 | C | 10 | 31 Mar. | 918 | 452 | 106 | 175 | 108 | 77 | 40 | — | — | — | 34 | — | — | — | 551 | 271 | 64 | 131 | 65 | 46 | 24 | 17 |
| 9 | N | 4 | 2 May | 882 | 499 | 374 | 142 | 176 | 131 | 139 | 440 | 118 | 110 | — | 60 | 40 | 38 | 870 | 390 | 310 | 85 | 152 | 109 | 112 | 12 |
| 10 | I | 4 | 9 Apr. | 860 | 455 | 166 | 76 | 72 | 56 | 59 | 500 | 110 | 40 | — | — | — | — | 903 | 358 | 130 | 16 | 13 | 34 | 51 | 12 |
| 11 | C | 4 | 18 Feb. | 837 | 504 | 342 | 508 | 328 | 166 | 194 | — | — | — | — | — | — | — | 502 | 302 | 205 | 305 | 197 | 100 | 116 | 26 |
| 12 | B | 4 | 18 Feb. | 832 | 324 | 207 | 108 | 90 | 90 | 65 | — | — | — | — | — | — | — | 499 | 194 | 124 | 65 | 54 | 54 | 39 | 12 |
| 13 | G | 4 | 25 Apr. | 829 | 589 | 245 | 198 | 176 | 135 | 155 | 300 | 70 | 30 | — | — | — | — | 504 | 408 | 170 | 119 | 106 | 81 | 93 | 14 |
| 14 | H | 3 | 28 Apr. | 826 | 1300 | 202 | 229 | 167 | 157 | — | 160 | — | 50 | — | — | — | — | 620 | 780 | 121 | 176 | 100 | 94 | — | Heifer |
| 15 | J | 3 | 9 Apr. | 796 | 466 | 283 | 171 | 76 | 49 | 51 | 38 | — | 24 | — | — | — | — | 507 | 280 | 189 | 103 | 46 | 29 | 32 | Heifer |
| 16 | K | 7 | 21 Mar. | 789 | 700 | 439 | 76 | 79 | 140 | 72 | 580 | 420 | 250 | — | — | 26 | — | 922 | 745 | 456 | 46 | 47 | 104 | 43 | 12 |
| 17 | D | 5 | 27 Mar. | 760 | 198 | 119 | 106 | 63 | 77 | — | 600 | 80 | 32 | — | — | — | — | 920 | 181 | 97 | 64 | 38 | 46 | — | 12 |
| 18 | G | 4 | 4 Apr. | 751 | 772 | 162 | 70 | 79 | 79 | 54 | 240 | 140 | 26 | — | — | — | — | 636 | 572 | 116 | 42 | 47 | 47 | 32 | 10 |
| 19 | C | 6 | 22 Feb. | 741 | 387 | 222 | 180 | 122 | 203 | 162 | — | — | — | — | — | 70 | 50 | 445 | 232 | 133 | 108 | 73 | 176 | 136 | 17 |
| 20 | E | 4 | 15 Mar. | 735 | 364 | 97 | 34 | 34 | 32 | 16 | 180 | 30 | — | — | — | — | — | 570 | 242 | 58 | 20 | 20 | 19 | 10 | 17 |
| 21 | K | 4 | 22 Mar. | 720 | 360 | 193 | 66 | 266 | 72 | 83 | 604 | 302 | 60 | — | 22 | — | — | 909 | 450 | 162 | 40 | 176 | 43 | 50 | 10 |
| 22 | B | 6 | 17 Feb. | 702 | 461 | 256 | 180 | 72 | 86 | 108 | — | — | — | — | — | — | — | 421 | 277 | 154 | 108 | 43 | 52 | 65 | 12 |
| 23 | D | 6 | 9 Mar. | 662 | 644 | 324 | 180 | 126 | 85 | — | 88 | 130 | 70 | 90 | — | — | — | 465 | 487 | 248 | 178 | 76 | 51 | — | 12 |
| 24 | F | 3 | 22 Mar. | 632 | 198 | 216 | 86 | 126 | — | 108 | 308 | 22 | 24 | — | — | — | — | 618 | 136 | 148 | 52 | 76 | — | 65 | Heifer |
| 25 | D | 5 | 5 Apr. | 616 | 288 | 266 | 110 | 50 | 61 | — | 360 | 52 | 90 | 30 | — | — | — | 648 | 213 | 229 | 89 | 30 | 37 | — | 21 |
| 26 | H | 3 | 29 Apr. | 615 | 468 | 283 | 184 | 162 | 90 | 72 | — | 32 | — | — | — | — | — | 369 | 306 | 170 | 110 | 97 | 54 | 43 | Heifer |
| 27 | O | 6 | 31 Mar. | 585 | 273 | 184 | 176 | 70 | 50 | 45 | — | — | 50 | 42 | — | — | — | 351 | 164 | 149 | 138 | 42 | 30 | 27 | 10 |
| 28 | O | 5 | 1 Apr. | 585 | 256 | 72 | 142 | 63 | 40 | 56 | 280 | 66 | 36 | 44 | — | — | — | 567 | 204 | 71 | 120 | 38 | 24 | 34 | 10 |
| 29 | O | 5 | 2 Apr. | 544 | 337 | 270 | 110 | 47 | 58 | 22 | 400 | 200 | 66 | 20 | — | — | — | 635 | 369 | 214 | 125 | 28 | 35 | 13 | 10 |
| 30 | C | 5 | 27 Feb. | 540 | 482 | 171 | 306 | 151 | 110 | 112 | 280 | 240 | 60 | 90 | 50 | 20 | — | 540 | 475 | 149 | 253 | 131 | 82 | 67 | 15 |
| 31 | A | 3 | 28 Feb. | 510 | 85 | 63 | 40 | 52 | 45 | 91 | 170 | — | — | — | — | — | — | 446 | 51 | 38 | 24 | 31 | 27 | 56 | 15 |
| 32 | J | 8 | 4 Apr. | 504 | 481 | 140 | 76 | 130 | 52 | 46 | 340 | 180 | 48 | — | 30 | — | — | 565 | 428 | 121 | 46 | 101 | 31 | 28 | 15 |
| 33 | N | 4 | 9 May | 505 | 414 | 90 | 126 | 432 | 241 | 104 | 34 | 36 | — | 46 | 52 | 21 | — | 329 | 276 | 54 | 111 | 299 | 164 | 62 | 12 |
| 34 | I | 5 | 29 Mar. | 490 | 475 | 212 | 144 | 90 | 97 | 58 | 302 | 240 | 86 | — | 24 | 24 | — | 528 | 471 | 194 | 86 | 73 | 97 | 35 | 12 |
| 35 | I | 5 | 30 Mar. | 486 | 162 | 126 | 106 | 72 | 77 | — | 220 | 60 | — | 24 | — | — | — | 461 | 144 | 76 | 82 | 43 | 46 | — | 12 |
| 36 | I | 11 | 11 Apr. | 484 | 128 | 86 | 81 | 68 | 54 | 36 | 100 | 26 | — | 20 | 24 | — | — | 367 | 97 | 52 | 64 | 60 | 32 | 22 | 12 |
| 37 | B | 6 | 5 Mar. | 469 | 202 | 110 | — | 38 | — | 54 | 80 | — | — | — | — | — | — | 344 | 121 | 66 | — | 23 | — | 32 | 9 |
| 38 | A | 7 | 15 Mar. | 468 | 162 | 212 | 68 | 72 | 70 | 101 | 220 | 56 | — | — | — | — | — | 451 | 141 | 127 | 41 | 43 | 42 | 61 | 9 |
| 39 | K | 8 | 25 Mar. | 455 | 774 | 270 | — | 117 | 90 | 76 | 480 | 840 | 120 | — | — | — | — | 670 | 1114 | 255 | — | 70 | 54 | 47 | 11 |
| 40 | C | 6 | 25 Feb. | 450 | 418 | 175 | 216 | 112 | 54 | 112 | — | — | 50 | 60 | 20 | — | — | 270 | 251 | 143 | 176 | 83 | 32 | 67 | 14 |
| 41 | C | 5 | 16 Apr. | 446 | 306 | 200 | 191 | 218 | 45 | 54 | 200 | 100 | 52 | 34 | 50 | — | — | 423 | 261 | 160 | 141 | 170 | 27 | 32 | 11 |
| 42 | [illegible] | 4 | 21 Mar. | 437 | 198 | 171 | 220 | 72 | 113 | 101 | 120 | 64 | 104 | — | — | — | — | 355 | 168 | 184 | 203 | 43 | 68 | 61 | 17 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 324 | 342 | 184 | 34 | 112 | 122 | — | 16 | 41 | — | — | — | 255 | 184 | 229 | 240 | 110 | 20 | 67 | 13 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 40 | 108 | [illegible] | [illegible] | 30 | 20 | [illegible] | [illegible] | 235 | 51 | 16 | 90 | 12 | 65 | 24 | 9 |
| [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 311 | 280 | 161 | 76 | 61 | 73 | 42 | 9 |

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [illegible] | [illegible] | [illegible] | [illegible] Apr. | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | 128 | 180 | [illegible] | [illegible] | — | — | — | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] | [illegible] |
| 49 | M | 6 | 30 Mar. | 400 | 198 | 275 | 101 | 90 | 76 | 72 | 88 | 54 | — | — | — | — | — | 308 | 161 | 165 | 61 | 54 | 46 | 43 | 9 |
| 50 | B | 6 | 25 Mar. | 392 | 319 | 108 | 103 | 119 | 112 | 65 | 88 | 70 | — | — | — | — | — | 303 | 245 | 65 | 62 | 71 | 67 | 39 | 10 |
| 51 | L | 6 | 2 Apr. | 382 | 184 | 230 | 101 | 94 | 54 | 41 | 220 | 80 | 86 | 30 | 26 | — | — | 399 | 163 | 205 | 84 | 77 | 32 | 25 | 9 |
| 52 | G | 3 | 24 Mar. | 374 | 216 | 175 | 146 | 54 | 86 | 108 | 86 | 50 | 30 | 34 | — | — | — | 291 | 169 | 126 | 114 | 32 | 52 | 65 | Heifer |
| 53 | G | 4 | 24 May | 374 | 346 | 103 | 171 | — | — | — | 480 | 86 | 54 | 32 | — | — | — | 596 | 274 | 120 | 128 | — | — | — | 17 |
| 54 | F | 4 | 2 May | 371 | 113 | 144 | 156 | 104 | 36 | 9 | 76 | — | — | 52 | — | — | — | 281 | 68 | 86 | 135 | 62 | 22 | 5 | 8 |
| 55 | H | 6 | 14 Apr. | 365 | 319 | 248 | 214 | 149 | 90 | 54 | 140 | 108 | 40 | 60 | 36 | — | — | 223 | 269 | 158 | 175 | 117 | 54 | 32 | 16 |
| 56 | C | 5 | 5 Mar. | 362 | 245 | 86 | 126 | 83 | 63 | 67 | 150 | 54 | — | — | — | — | — | 334 | 189 | 52 | 76 | 50 | 38 | 40 | 17 |
| 57 | A | 8 | 12 Mar. | 351 | 184 | 108 | 50 | 58 | 50 | 108 | 230 | 20 | — | — | — | — | — | 389 | 126 | 65 | 30 | 35 | 30 | 65 | 8 |
| 58 | L | 10 | 4 Apr. | 346 | 178 | 54 | — | 68 | 45 | 38 | 130 | 150 | 24 | — | 26 | — | — | 308 | 223 | 51 | — | 61 | 27 | 23 | 10 |
| 59 | G | 3 | 20 May | 342 | 246 | 135 | 77 | 157 | 54 | 72 | 40 | 26 | — | — | — | — | 36 | 236 | 168 | 81 | 46 | 94 | 32 | 71 | Heifer |
| 60 | J | 5 | 4 Apr. | 342 | 562 | 409 | 409 | 162 | 59 | 68 | 200 | 180 | 130 | 86 | — | — | — | 360 | 476 | 346 | 312 | 97 | 35 | 41 | 13 |
| 61 | J | 4 | 9 Apr. | 342 | 381 | 396 | 130 | 142 | 70 | 56 | 80 | 28 | 120 | 36 | — | — | — | 267 | 250 | 330 | 106 | 85 | 42 | 34 | 13 |
| 62 | K | 6 | 22 Mar. | 337 | 347 | 421 | 158 | 288 | 90 | 86 | 580 | 292 | 380 | 70 | 128 | — | — | 650 | 434 | 570 | 149 | 272 | 54 | 52 | 18 |
| 63 | L | 6 | 5 Apr. | 337 | 682 | 324 | 86 | 90 | 122 | 38 | 240 | 480 | 180 | 20 | — | — | — | 388 | 780 | 334 | 67 | 54 | 73 | 23 | 9 |
| 64 | H | 5 | 10 Mar. | 328 | 436 | 256 | 90 | 106 | 184 | 72 | 62 | 20 | 42 | — | — | — | — | 145 | 277 | 186 | 54 | 64 | 110 | 43 | 14 |
| 65 | B | 5 | 3 Mar. | 324 | 423 | — | 124 | 110 | — | 36 | 150 | 194 | — | — | — | — | — | 310 | 401 | — | 73 | 66 | — | 22 | 8 |
| 66 | E | 4 | 1 Mar. | 306 | 288 | 56 | 135 | 90 | 72 | 86 | 78 | — | 8 | — | — | — | — | 244 | 240 | 34 | 81 | 54 | 43 | 52 | 12 |
| 67 | F | 5 | 8 Mar. | 302 | 86 | 76 | 139 | 68 | 83 | 45 | 20 | — | — | — | — | — | — | 197 | 52 | 46 | 83 | 41 | 50 | 27 | 32 |
| 68 | B | 7 | 6 Mar. | 275 | 234 | — | 221 | 101 | 108 | — | 38 | — | — | — | — | — | — | 195 | 140 | — | 133 | 61 | 65 | — | 8 |
| 69 | A | 7 | 27 Mar. | 270 | 117 | 79 | 40 | 27 | — | 76 | 600 | 80 | — | 20 | — | — | 60 | 626 | 132 | 47 | 40 | 22 | — | 92 | 4 |
| 70 | E | 5 | 8 Mar. | 266 | 396 | 86 | 117 | 85 | 63 | 54 | 40 | 54 | — | — | — | — | — | 190 | 280 | 52 | 70 | 51 | 38 | 32 | 26 |
| 71 | D | 5 | 18 Feb. | 261 | 234 | 171 | 180 | 277 | 72 | — | — | — | — | — | — | — | — | 157 | 140 | 103 | 108 | 166 | 43 | — | 8 |
| 72 | H | 5 | 10 Apr. | 252 | 166 | 122 | 61 | 63 | 59 | 41 | — | — | — | — | 34 | — | — | 151 | 100 | 73 | 37 | 64 | 35 | 25 | 11 |
| 73 | E | 8 | 14 Mar. | 248 | 211 | 86 | 144 | 72 | 36 | 22 | 120 | — | — | — | — | — | — | 248 | 127 | 52 | 86 | 43 | 22 | 13 | 17 |
| 74 | G | 4 | 20 May | 248 | 275 | 121 | 162 | 146 | 67 | 54 | 52 | 28 | 26 | — | — | — | — | 189 | 186 | 93 | 97 | 88 | 70 | 32 | 6 |
| 75 | D | 5 | 7 Mar. | 238 | 292 | 50 | 155 | 110 | 58 | — | 60 | 56 | — | — | 36 | — | — | 189 | 219 | 30 | 93 | 94 | 35 | — | 8 |
| 76 | H | 3 | 5 Apr. | 234 | 171 | 166 | 92 | 40 | 86 | 36 | 20 | — | — | — | — | — | — | 156 | 103 | 100 | 55 | 24 | 52 | 22 | Heifer |
| 77 | F | 5 | 17 Feb. | 230 | 180 | 225 | 108 | 72 | 54 | 103 | — | — | — | — | — | — | — | 138 | 108 | 135 | 65 | 43 | 32 | 62 | 6 |
| 78 | C | 5 | 12 Mar. | 230 | 130 | 43 | 76 | 90 | 86 | 61 | 230 | 36 | — | — | — | — | — | 316 | 106 | 26 | 46 | 54 | 52 | 37 | 10 |
| 79 | A | 4 | 13 Mar. | 212 | 68 | 290 | 97 | 65 | 61 | 50 | — | — | 160 | — | — | — | — | 127 | 41 | 297 | 58 | 39 | 37 | 30 | 9 |
| 80 | B | 5 | 12 Mar. | 211 | 135 | 40 | 76 | 108 | 67 | 74 | — | — | — | — | — | — | — | 127 | 81 | 24 | 46 | 65 | 40 | 44 | 8 |
| 81 | D | 7 | 17 Mar. | 185 | 122 | 112 | 54 | 84 | 94 | — | — | — | — | — | 34 | — | — | 111 | 73 | 67 | 32 | 78 | 56 | — | 10 |
| 82 | D | 11 | 12 Apr. | 180 | 140 | 94 | 74 | 74 | 85 | — | 34 | 36 | 40 | 40 | — | — | — | 134 | 112 | 87 | 75 | 44 | 51 | — | 12 |
| 83 | A | 4 | 18 Apr. | 178 | 72 | 76 | 85 | 106 | 18 | 41 | 260 | 90 | 40 | 22 | 34 | — | — | 308 | 116 | 76 | 59 | 90 | 11 | 25 | 5 |
| 84 | D | 3 | 10 Apr. | 178 | 85 | 54 | 67 | 61 | 40 | — | 44 | — | — | — | 32 | 30 | — | 141 | 51 | 32 | 40 | 62 | 47 | — | 8 |
| 85 | B | 5 | 17 Mar. | 171 | 130 | — | 88 | 126 | 106 | 94 | — | — | — | — | — | — | — | 103 | 88 | — | 53 | 76 | 64 | 56 | 6 |
| 86 | E | 5 | 15 Mar. | 167 | 72 | 126 | 54 | 74 | 59 | 124 | 30 | — | — | — | — | — | — | 123 | 43 | 76 | 32 | 44 | 8 | 74 | 8 |
| 87 | F | 5 | 15 Mar. | 166 | 176 | 180 | 86 | 77 | 54 | 72 | — | — | — | — | — | — | — | 100 | 106 | 108 | 52 | 46 | 32 | 43 | 19 |
| 88 | D | 3 | 18 May | 164 | 234 | 490 | 101 | 90 | 90 | 124 | — | — | 48 | 24 | 20 | — | — | 98 | 110 | 331 | 79 | 70 | 54 | 74 | Heifer |
| 89 | G | 5 | 14 May | 142 | 157 | 113 | 130 | 54 | 72 | 77 | 220 | 116 | 32 | 46 | — | — | 34 | 255 | 179 | 93 | 113 | 32 | 43 | 72 | 10 |
| 90 | A | 4 | 22 Apr. | 142 | 50 | 113 | 50 | 22 | 54 | 125 | — | — | — | — | — | — | — | 85 | 30 | 93 | 30 | 43 | 32 | 75 | 20 |
| 91 | E | 5 | 22 Feb. | 135 | 126 | 119 | 77 | 41 | 27 | 36 | — | — | — | — | — | — | — | 81 | 76 | 70 | 46 | 25 | 16 | 22 | 16 |
| 92 | D | 7 | 22 Mar. | 130 | 131 | 72 | 88 | 65 | 81 | — | 108 | 100 | 22 | 48 | 20 | 26 | — | 161 | 156 | 60 | 90 | 55 | 69 | — | 8 |
| 93 | A | 4 | 10 Mar. | 126 | 378 | 176 | 130 | 54 | 40 | 50 | 22 | 90 | 62 | — | — | — | — | 82 | 296 | 154 | 78 | 32 | 24 | 30 | 8 |
| 94 | L | 6 | 4 Apr. | 122 | 536 | 97 | 70 | 56 | 36 | 40 | 70 | 240 | 26 | — | — | — | — | 120 | 507 | 78 | 42 | 34 | 22 | 24 | 17 |
| 95 | E | 5 | 11 Mar. | 112 | 526 | 63 | 54 | 40 | 36 | 27 | 44 | 128 | — | — | — | — | — | 102 | 415 | 38 | 32 | 24 | 22 | 16 | 19 |
| 96 | E | 4 | 15 Mar. | 106 | 49 | 76 | 77 | 36 | 52 | 30 | 26 | — | — | — | — | — | — | 84 | 29 | 46 | 46 | 22 | 31 | 22 | 3 |
| 97 | G | 4 | 8 May | 106 | 90 | 65 | 63 | 76 | 43 | 94 | — | — | — | — | — | — | — | 64 | 54 | 39 | 38 | 46 | 26 | 95 | 8 |
| 98 | G | 5 | 6 Apr. | 86 | 94 | 90 | 38 | 72 | 63 | — | — | 52 | 34 | — | — | — | — | 52 | 96 | 80 | 23 | 43 | 38 | — | 8 |
| 99 | E | 7 | 23 Feb. | 76 | 108 | 58 | 36 | 27 | 18 | 27 | — | — | — | — | — | — | — | 46 | 65 | 35 | 22 | 16 | 11 | 16 | 8 |
| 100 | F | 4 | 23 Apr. | 59 | 58 | 41 | 18 | 18 | 13 | 54 | — | — | — | — | — | — | — | 35 | 35 | 25 | 11 | 11 | 8 | 32 | 3 |

content of vitamin A than that of other cows. This is not borne out in the present experiment—the values for the colostrum of heifers being distributed evenly over Table I (see Fig. 2). The age of the cow does not seem to affect the vitamin A content of the colostrum to any significant degree. The vitamin A content of the colostrum can remain high even

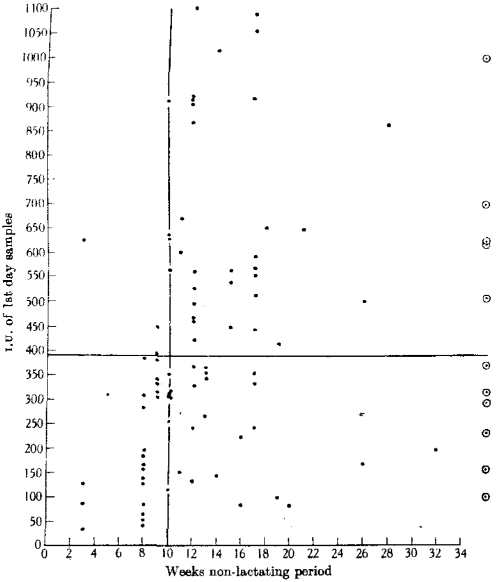


Fig. 2. ⊙ = Heifer.

after several lactations, as in the case of nos. 7 and 8. The influence of age in affecting the vitamin content of the colostrum must be considered in conjunction with the length of the "dry" or non-lactating period between successive calvings. It will be seen from the last column of Table I that most of the cows which possessed a value for the vitamin A content of their colostrum of above 392 I.U. per 100 ml. (the arithmetical

mean of the 100 samples) had a non-lactating period ranging from ten weeks to four months and sometimes longer, whilst those showing the lowest values had one of usually under ten weeks. This is still more clearly shown in Fig. 2—a scatter diagram of the international units graphed against the weeks of the non-lactating period. There are a few exceptions in Table 1, but nos. 67 and 70 can be accounted for, as they were in poor health at the time of calving, and nos. 94 and 95 had trouble at calving and would really be much higher up the table if the second samples were taken into account. Since it is usually assumed that the vitamin A content of the colostrum depends directly on the liver reserves, it can readily be understood that the longer the non-lactating period, the greater the chance of the content being high. Moore (1932) has shown how quickly the reserves of vitamin A in the liver, accumulated whilst the cows were at grass, could be used up during stall feeding on artificial food, almost devoid of carotene. If, therefore, the lactation period is prolonged to such an extent as to deplete these reserves, and time is not allowed to build them up again before the next parturition, it is to be expected that the vitamin A content of the colostrum would be low. The deficiency of carotene in the foodstuffs comprising winter rations is fully reflected by the lack of carotene in the fifth, sixth and seventh day samples when milk has started to be secreted. The low values for the fifth, sixth and seventh samples also show how completely the carotene reserves are used up by the colostrum during the first two or three days after calving.

It is possible, therefore, that an explanation of the wide range found in the vitamin A content of the colostrums is a large variation in the reserves of vitamin A in the livers of the cows near the date of parturition, and that this is directly influenced by the length of time allowed between the previous lactation and the date of parturition. Moreover, if this explanation is true, a simple practical method to ensure a high vitamin A content of the colostrum would be to allow a non-lactating period of not less than three months. The economics of such a practice as compared with the feeding of a vitamin A concentrate would have to be investigated.

## SUMMARY

1. The vitamin A content of the colostrum of one hundred cows, kept under the same conditions of dietary and management, was found to range from 1181 to 35 I.U. per 100 ml.

2. It has been established that this wide range was not due to any

differences in dietary, breed or date of calving in animals kept under the same conditions of management.

3. It is suggested that the vitamin A content of the colostrum may be materially affected by the length of the non-lactating period between successive calvings.

We are indebted to Dr J. Russell Greig, Director of the Institute, for his kind interest and assistance in regard to the planning of the experiment, and to Prof. A. J. Clark, Edinburgh University, for his valuable criticism and advice.

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# INVESTIGATIONS ON CLOVER SICKNESS

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The fact that land which frequently bears a crop of clover becomes rapidly unfit to bear further crops of this plant is a very old observation in all countries where clover is grown. In England, Arthur Young (1804) called attention to it and to the fact that farmers were accustomed to call this unfitness "clover sickness". The cause of this soil condition has been a matter of careful study since early in the nineteenth century, and some of the most interesting observations on the subject were made by Lawes & Gilbert (1860) in the years preceding 1860. They showed that the lack of capacity to grow clover did not seem to be connected with any deficiency in the principal specific plant foods in the soil, and the only contrary evidence was the fact that when a rich garden soil was substituted for the ordinary field soil, it was possible to grow clover for a much longer period without failure (Gilbert, 1871). The final conclusion of Lawes & Gilbert as to the so-called clover sickness was that the only means of ensuring a good crop of red clover is to allow some years to elapse before repeating the crop on the same land.

From about 1880, the opinion became more and more general, though by no means universal, that the loss of capacity to produce a clover crop on certain soils was due in almost all cases to the attack of parasitic diseases, usually eelworms or fungi, and interest has chiefly centred since that time in devising methods of checking the attack of these parasites. This attitude is well represented in the two best general accounts of clover sickness hitherto published, one in England by Amos (1918), and the other in the United States by Pieters (1924). Both of these are inclined to attribute the failure to grow crops of red clover to one of a number of fungi or to certain parasitic eelworms. The most important fungi said to be involved were *Sclerotinia trifoliorum*, in England, and several, among which was *Colletotrichum trifolii*, in the United States. The parasitic eelworm which has been most closely connected with the condition is *Anguillulina dipsaci*. The most generally accepted opinion has been perhaps best expressed by Pieters when he says: "The soil does not become sick of clover, but the clover itself may become sick, as indeed it does when attacked by specific diseases or when the soil

*conditions are not suited to its growth. Clover fails either because the soil is unsuited to its development, because it is attacked by a fungus, or an insect, or a nematode, or because of the use of seed of strains not adapted to the climatic conditions."*

He then proceeds to list the causes of clover failure, as (1) unsuitable soil conditions, (2) unadapted or poor seed, (3) poor methods of seeding, (4) diseases and insects, and (5) wrong fall treatment in the first year. To this list Amos (1918) adds (*a*) lack of lime in the soil, (*b*) lack of inoculation with the proper nodule bacteria, and (*c*) possibly the excretion of toxic or poisonous substances by the clover roots. Since the two papers quoted were written, this list has been increased by the suggestions that clover failure may be due to the presence of an unsuitable strain of nodule bacteria, or to the presence of a bacteriophage.

There is little doubt that what is called clover sickness is associated with a multitude of active agents, but the question which has interested the writer is to ascertain whether any of these can be considered as the prime cause of the lack of vigour in growing clover plants, or whether they are merely the active agents in killing an already weakened plant. To examine the matter it was necessary to take a single case where clover would not grow vigorously and, if possible, to eliminate all the casual and incidental attacks, and so to find whether something remains which is present even in the absence of the active agents to which the affection has been attributed.

## Agricultural meaning of "clover sickness"

Before giving an account of the observations and experiments which I have made during the last six years, I should like to consider what is really meant by a farmer when he speaks of his land being "clover sick". In my experience, such a man may understand one of three things. First, he may mean that there is great difficulty in establishing a stand of clover or, at any rate, a crop of properly sized plants. Secondly, he may mean that, after a stand of clover is established, the clover dies either generally or in patches, chiefly during the winter after the clover has been planted. Thirdly, he may mean that, though he gets the ground more or less covered with clover plants, these never grow to a normal size, but remain small, sometimes very small, and never yield a satisfactory crop.

The failure to establish a plant, and the death of the plant after it has been established (usually in the succeeding winter), seem often to be connected either with deficiencies of the land which can be very

easily ascertained—like the absence of a good seed bed, the absence of lime, the lack of drainage, the too great acidity of the soil—or with the attack of specific parasites, when the parasite involved in each case can usually easily be determined. I have had cases where red clover in the field has been almost a complete failure, but where, given suitable and favourable conditions, the same soil has given me a first-class crop of the same plant. In these cases it was clear that the conditions of planting had been unfavourable, and no specific deficiency of the soil nor any parasitic disease was involved. In other cases where the plants died during the winter following sowing of the seed, it has been quite easy to see the attack of a parasitic fungus spreading in circles, so that the roots of the plants were afterwards found in a rotted condition.

The other meanings of clover sickness referred to above are not nearly so easy to clear up. The incapacity of the plants to grow normally on the soil either in the autumn of the year of sowing, even when the conditions were made as favourable as possible, or again in the following year when the crop should be ready for cutting, is not nearly so obviously connected with specific parasites or with deficiencies of soil preparation or treatment, and yet this is, I think, what is most frequently meant by a farmer who says that his land is clover sick. In other words, in the farmer's mind, it is not the death of the plants which he usually calls "clover sickness" but the absence of normal and luxuriant growth, whether the plants die or survive. From the point of view of the present study, I should, in fact, like to define clover sickness of soil as being the incapacity of the land to produce a properly sized plant of clover, and its intensity would be measured by the relative size of plant produced in a definite time compared with that produced in a normal, healthy soil of the same kind with similar manuring. Of course, such reduction in size may be produced by many causes, including all or nearly all the parasites which have been found in clover grown on clover-sick land. In the latter case, however, there would be definite symptoms connecting the plants with the specific parasite. Clover sickness, however, from an agricultural point of view, seems to consist in the reduction in the size of the plants, and this reduction is a measure of its intensity, from whatever origin it may arise.

## Experimental work

The present study was the result of the failure of red clover on a block of land in Stackyard Field at Woburn in 1931. This land had been under a four-course rotation, usually, but not always, containing clover, at

least since 1877. In the three previous clover years, the crop had been quite reasonably good. Thus in

1919, red clover gave 2 tons of hay per acre in two cuttings.
1923, red clover gave $2\frac{1}{2}$ tons of hay per acre in two cuttings.
1927, mixed clovers (red, alsike, and trefoil) gave 3 tons of hay per acre in one cutting.

There was, in fact, no history of clover failure. But, in 1931, the red clover, which had been undersown in the previous crop of barley, came up very patchy, was very poor during the winter, and showed no sign of growing up to the end of March. It was then ploughed up and, after an interval, tares were sown in its stead. It was at this stage that the soil was taken and placed in a series of deep pots, and the experiments have been carried on with it ever since. The pots were arranged so that experiments with the soil which had proved itself sick for red clover, could be sown with red clover, alsike clover, Dutch white clover and crimson clover (*Trifolium incarnatum*).

The clover sickness, so far as red clover was concerned, was quickly evident even with the first crop of this plant in such pots, but though the majority of the plants remained small, were obviously unhealthy and were affected with pin-point red spotting of the leaves, and ultimately died, a certain number (ten out of thirty-five) gave good and well-grown plants, though almost all died before the end of 1931. Alsike clover, Dutch white clover and crimson clover grew very well without any sign of the reduction in size of the plants. A secoud crop was sown in each pot in the following year when almost all the red clover plants now showed great reduction in size, and the alsike clover showed irregularities similar to those which had been noticed with the red clover in the first year. The Dutch white clover and the crimson clover still showed a perfectly healthy and well-grown crop. The same crops were again grown in the same pots in the following year, when all the alsike and red-clover plants showed the reductiou in size, and now the Dutch white clover showed similar symptoms, though the crimson clover still showed healthy growth. The last-named clover succumbed in the next year, when the plants refused to grow at all. We thus obtained soil which had successively become incapable of growing healthy plants of red clover (1931), alsike clover (1932), Dutch white clover (1933) and crimson clover (1934).

Having prepared in this manner soil which was thoroughly clover sick, in the sense that clover either refused to grow at all (crimson clover) or gave small undersized plants which were liable to die (all the other

clovers), it has been possible to see on the one hand what supposed causes of clover sickness can be eliminated in this case, and to find what methods can be used to modify the soil so as to produce a well-grown healthy plant of each clover. For the purposes given, the clover crops have been continued, one after another, in the same pots right up to 1937, except in the case of crimson clover, which will be dealt with separately.

First, we may eliminate altogether any question of failure or dwarfing of the crops due to faulty preparation of the soil. Similar preparation of the pots has been done in each case, and yet, as will be seen later, there has been every variation of size and vigour in the clover plants obtained, due to several treatments.

Secondly, there have not usually been clear signs of the absence of nodule organisms. Even in the crop taken in the spring of 1937, nodules were found abundant on the roots of all plants, both on the main roots and on the rootlets, except in two or three cases of plants grown on the subsoil only. Even in the smallest plants grown on surface soil, there were nodules on both types of roots whatever the treatment adopted and whatever the condition of the soil so far as clover sickness is concerned.

Thirdly, it was suggested at one time of the experiment that the failure of the clovers was due primarily to poverty of the soil. To test this point, a crop of mustard was taken instead of clover on one pot of each set in 1935 (spring). In all cases the mustard grew healthily and vigorously, and showed no signs of soil exhaustion. Further, manuring of the soil with calcium carbonate (20 g. per pot, equal to 4·4 tons of calcium carbonate per acre) and potassium phosphate ($KH_2PO_4$ 0·77 g. per pot equal to 1·75 cwt. phosphoric acid and 1·2 cwt. potash per acre) in 1935 did not cause normally sized plants to be produced, though they were not obviously unhealthy.

Fourthly, the soil had a $p$H value of 6·0–6·5 which, though lower than is considered the best for any of the clovers in question, is quite capable of giving healthy and normal plants. In the long period for which we have the history of this soil in the field, there have been several applications of basic slag and of lime, so that the absence of lime cannot be considered a possible cause of the clover sickness.

Fifthly, very careful leaching of the soil did not, in the case of crimson clover, cause any improvement in the succeeding crop, indicating that it was not any accumulation of soluble material, excretory or otherwise, which caused the crop failure.

Lastly, no sign of *Sclerotinia trifoliorum* has ever been found in the pots under discussion. This fungus is common in the area from which the soil was taken, and its attack has frequently been noticed in the field. But from the history of these pots, it is quite clear that we can have the dwarfing of the plants without the occurrence of this fungus at all.

Mildew (*Erysiphe communis*) has been common on the clovers throughout the experiment, but it has never led to symptoms which at all resemble the dwarfing of the plants characteristic of clover sickness. It has often killed the foliage, but never dwarfed the plants seriously.

We have thus got all the characteristic signs of what is generally called clover sickness without the presence of any of the causes which were listed above. It remains to see what other of the supposed causes are actually present, and then to ascertain whether the effect of various treatments on the dwarfing of the plants is paralleled by their effect on the supposed cause.

The principal supposed parasitic cause of the affection known as clover sickness, which is present in the case we are examining, is the eelworm, *Anguillulina dipsaci*, which has been found on plants grown on this soil in many, but not by any means in all, cases, and the effect of the various treatments has been studied with reference to this parasite. In this study we have had the invaluable co-operation of Dr T. Goodey, of the Institute of Agricultural Parasitology, St Albans, who has examined the plants and the soil under many conditions and supplied notes of the eelworm population.

## Treatments employed

The treatments which have been studied have been:

(1) The addition of calcium carbonate and potassium phosphate, to ensure that sufficient mineral plant food was present.

(2) The addition of potassium nitrate, to ensure that soluble nitrogen was present, even if the root nodules are not serving their purpose.

(3) The heating of the soil so as partially to sterilize it, either in the moist or the dry condition.

(4) The addition of farmyard manure in large quantity, in order to compare the results with those obtained by Gilbert on rich garden soil.

The effect of each of these treatments on the luxuriance of the plants and on the content of parasitic eelworms in the plants grown will now be considered.

*(1) The effect of mineral manures*

In the spring of 1935, one of two pots of soil (which had been partially sterilized in 1934) was treated with 20 g. calcium carbonate and 0·77 g. $KH_2PO_4$ per pot, mixed with the top 6 in. of the soil, equivalent to a dressing of 4·4 tons of calcium carbonate, 1·75 cwt. of phosphoric acid and 1·2 cwt. potash per acre.

The general result of the manuring on the luxuriance of the clover plants, of all three kinds, was very slight, and the weight per plant (after growing from 13 September 1935 to April 1936) was very similar to that in the pots which had not been manured but had been heated similarly in 1934. The actual weights of the plants in the comparable pots were:

Table I

| | Weight per green plant | |
|---|---|---|
| | Pot not manured g. | Pot manured with $CaCO_3$ and $KH_2PO_4$ g. |
| Broad red clover | 0·30 | 0·20 |
| Alsike clover | 0·05 | 0·15 |
| Dutch white clover | 0·25 | 0·15 |

It will be seen that all the plants were very small, and that the manuring with calcium carbonate and potassium phosphate in the manner described has not brought back the luxuriance of any of the clovers.

Further, in the plants from all these pots, Goodey was unable to find any *Anguillulina dipsaci* in the crop reaped in April 1936, in spite of very intense dwarfing of the plants, the symptom of clover sickness in the sense in which I have defined it.

Another crop was sown on these pots on 22 April 1936, and the lack of value of the manurial treatment to affect the dwarfing of the plants was amply proved.

Table II shows the relative size of the plants obtained in the pots treated with fertilizers as compared with those which had received no treatment since 1931, when harvested in July 1936.

Table II

| | Height of plants | | Weight per plant (mean) | |
|---|---|---|---|---|
| | No treatment cm. | With fertilizers cm. | No treatment g. | With fertilizers g. |
| Broad red clover | 9–11 | 5 | 0·9 | 0·3 |
| Alsike clover | 5–1½ | 5–8 | 0·03 | 0·27 |
| White clover | 10½–5½ | 5 | 0·29 | 0·17 |

With red clover, the pot to which fertilizer was added was actually the worst of the series, so far as size of plants was concerned; with alsike clover the size of the plants was lower than in any case except where the soil had never had any treatment whatever; and with Dutch white clover, the plants were very small and no larger than in any pot of the series.

It is quite clear, in fact, that with the type of clover sickness with which we are dealing, no material improvement in the character of the plants has been obtained by the use of the mineral manures specified.

### (2) *The effect of potassium nitrate*

Nitrates are not usually very suitable as manures for clovers but, with the object of ascertaining whether the lack of power to procure the nitrogen that the plants need from other sources was the trouble, this fertilizer was added in the autumn of 1936 when a new crop of each clover was sown. Unfortunately, owing to an accident, I can only give the results with red clover. But in this case there was no evidence that the size of the crop was at all benefited by the addition of 0·5 g. nitrogen per pot (equal to 244 lb. nitrogen per acre). In fact, the plants were very diminutive, and after growing from 8 August 1936 to 4 March 1937, only weighed 0·77 g. per green plant, while those without any treatment from the beginning of the experiment in 1931, weighed 0·75 g. per green plant.

Both sets of plants contained many *Anguillulina dipsaci*, with typical symptoms of the disease caused by this eelworm. It is quite clear that the addition of nitrate of potash, i.e. of available nitrogen in abundance, was neither capable of reducing the attack of the eelworm nor of rendering the soil capable of growing the clover to a normal size.

### (3) *The effect of heating the soil to* 60–70° C.

It is already well known that by completely sterilizing the soil, eelworms can be got rid of completely, though this treatment would be impracticable in the case of field soils. But the effect of a partial sterilization of the soil, so as to kill the actual active eelworms, though not the more resistant ones, was at least interesting. Such partial sterilization has been done, by several methods, since 1933.

The first attempt was with the soil from the top 6 in. only. This was removed in the early part of 1933, dried and then heated to 60–70° C. for 2 days. The results of this method were very slight. The weights of the plants and their condition relative to *Anguillulina dipsaci* was as follows:

Table III

| | Weight of plants per pot g. | Condition of plants relative to eelworms |
|---|---|---|
| Red clover | | |
| Unheated | 13·2 | Few in one pot: many in the other |
| Heated | 12·7 | Fair numbers |
| Alsike clover | | |
| Unheated | 19·0 | Few in one pot: many in the other |
| Heated | 24·8 | No *Anguillulina* |
| Dutch white clover | | |
| Unheated | Negligible | ? |
| Heated | 17·9 | No *Anguillulina* |

It seems evident that except with the Dutch white clover the heating of the dried soil to the temperature mentioned (60–70° C.) is not effective as a means of seriously reducing the incapacity of the soil to grow normally any of these clovers, and cannot even be relied upon to reduce materially the number of eelworms which get into the plants. In this case, however, it must be noticed that only the top 6 in. of the soil were heated, and that the soil was dried before heating. The eelworms may have re-entered the soil from the lower layers, or they may have been able completely to resist the dry heating.

The next step was to see whether there was any effect if the whole of the soil in each of the pots was heated, the heating being done while the soil was moist. The whole of the soil in two pots with each variety of clover was, therefore, taken out, immediately transferred to a room at over 60° C. and kept at this temperature for 2 hours when it was again filled into the pots, which had, in the meantime, been scalded. One of the pots, with each variety, was then reinfected with eelworms by working into the soil air-dry material known to contain abundance of *Anguillulina dipsaci*. After keeping the heated soil, both with and without re-infection, for 3 days in a moist condition, they were again sown with the respective types of clover. Two crops were then grown in succession with the following results:

BROAD RED CLOVER

*1st Crop* (sown 17 March 1935)

No treatment. The plants that grew were small but apparently healthy.

Heated to 60–70° C. Well-grown healthy plants.

Heated to 60–70° C. and reinfected. Good plants, but not quite so well grown as those in the last case.

*2nd Crop* (sown 8 August 1935)

No treatment. Plants small, unhealthy and died before November. (Good numbers of *Anguillulina dipsaci*, and a few other eelworms, notably *Cephalobus* sp. and *Plectus* sp.)

Heated to 60–70° C. Some plants died but remainder vigorous, well grown and healthy. (In dead plants a few *Cephalobus striatus* and *Anguillulina dipsaci*: in living plants, *Anguillulina dipsaci* in large numbers.)

Heated to 60–70° C. and reinfected. Some plants died but remainder vigorous, well grown and healthy. (In dead plants there were a few *Anguillulina dipsaci*, but *Cephalobus rigidus* was plentiful; in living plants, *Anguillulina dipsaci* was found in enormous numbers while *Cephalobus rigidus* was very plentiful.)

ALSIKE CLOVER

*1st Crop* (sown 17 March 1935)

No treatment. Plants small and very unhealthy.

Heated to 60–70° C. Plants apparently healthy, but small.

Heated to 60–70° C. and reinfected. Plants apparently quite healthy, but small.

*2nd Crop* (sown 8 August 1935)

No treatment. All plants died except one, which was big and vigorous. (*Anguillulina dipsaci* in large numbers, *Cephalobus rigidus* many.)

Heated to 60–70° C. Healthy, vigorous plants. (A few *Anguillulina dipsaci*, several *Cephalobus rigidus*, and a few *Aphelenchus avenae*.)

Heated to 60–70° C. and reinfected. Healthy, vigorous plants, though not quite as vigorous as the last. (Large numbers of *Anguillulina dipsaci*, many *Cephalobus rigidus*.)

DUTCH WHITE CLOVER

*1st Crop* (sown 17 March 1935)

No treatment. Plants were numerous, but small and very uneven in size. No specific disease.

Heated to 60–70° C. Plants uneven, but apparently healthy.

Heated to 60–70° C. and reinfected. The best pot of white clover.

*2nd Crop* (sown 8 August 1935)

No treatment. All plants became very unhealthy and several died. Little or no growth on any plant and few green leaves. (In dead plants, *Anguillulina dipsaci*, a few; *Aphelenchus avenae*, a few; *Cephalobus*, good numbers; *Rhabditis monohystera*, a few: in living plants, *Anguillulina dipsaci*, a few; *Aphelenchus avenae*, a few; *Cephalobus striatus*, several; *Aphelenchus parietinus*, one or two.)

Heated to 60–70° C. All plants healthy and vigorous. (*Anguillulina dubia*, several; *Aphelenchus avenae*, a few; *Cephalobus persegnis*, one or two; *Cephalobus rigidus*, one or two.)

Heated to 60–70° C. and reinfected. All plants healthy, but not very well grown. (*Anguillulina dipsaci*, a few; *Cephalobus rigidus*, plentiful.)

Two facts emerge from these results: the first is that there is a very marked recovery of the soil as a result of heating the moist soil to a temperature of 60–70° C. On such a soil there is no difficulty in getting

well-grown apparently healthy plants though, in the case of the alsike clover, even then the plants were classed as small. The attempted reinfection of the soil by working in dead clover plants grown on this clover-sick soil was quite ineffective in re-establishing the incapacity of the soil to grow clover.

The second point is the fact that there seems little relationship between the presence of the known parasitic species of nematode and the size or apparent vigour of the plants. In the case of broad red clover, in particular, the vigorous plants which remained contained *Anguillulina dipsaci* in large or enormous numbers.

Further crops of the same clovers were again grown for two further seasons, and it was evident that the reinfection of the soil with plants containing many eelworms was entirely ineffective in creating more clover-sick conditions than existed without it. But the effect of the heating of the soil in improving the soil conditions rapidly passed off. While in the first of these additional crops, there was a fair growth in the treated pots, usually far better than in those where no treatment had been attempted (except with alsike clover), in the second crop (reaped in April 1936) the plants were diminutive, and contrasted strongly with those grown in other pots of soil which had been similarly heated immediately before planting. This contrast is shown by the following records of weight per plant in the green condition.

Table IV

| | Weight per green plant (spring 1936) g. |
|---|---|
| Broad red clover | |
| No treatment since 1931 | No plants |
| Heated to 60–70° C. (spring 1934) | 0·30 |
| Heated to 60–70° C. (spring 1934) and reinfected | 0·20 |
| Heated to 60–70° C. (spring 1935) | 1·05 |
| Alsike clover | |
| No treatment since 1931 | 0·005 |
| Heated to 60–70° C. (spring 1934) | 0·05 |
| Heated to 60–70° C. (spring 1934) and reinfected | 0·15 |
| Heated to 60–70° C. (spring 1935) | 1·10 |
| Dutch white clover | |
| No treatment since 1931 | 0·05 |
| Heated to 60–70° C. (spring 1934) | 0·25 |
| Heated to 60–70° C. (spring 1934) and reinfected | 0·15 |
| Heated to 60–70° C. (spring 1935) | 0·005 |

Thus we see that the effect of the heating a year previousl, seems to have almost entirely disappeared in this crop, the plants being almost as small as with the untreated soil. The fresh heating of the soil in 1935 still shows

itself capable of giving plants four or five times as large, except in the case of the Dutch white clover. The different behaviour of the latter crop in the present case is as yet quite unexplained.

A still more striking result was found when the plants so grown were examined by Goodey for parasitic eelworms. In no case were these found, except with the red clover grown on soil heated in 1934, when a few individuals of *Anguillulina dipsaci* were discovered. In all other cases no parasitic eelworms were found in the plant at all, and yet the characteristic dwarfing of the plants was most obviously present.

The improvement of the plants after the heating in 1935 very quickly passed off as the following weights of the plant produced in spring and autumn sowings of 1936 and spring of 1937 show:

Table V

| | Weight per plant | | |
|---|---|---|---|
| | Spring sowing 1936 g. | Autumn sowing 1936 g. | Spring sowing 1937 g. |
| *Broad red clover* | | | |
| *No treatment since 1931* | 0·92 | 0·75 | 2·3 |
| *Heated to 60–70° C. (spring 1935)* | 4·71 | 5·10 | 2·3 |
| Alsike clover | | | |
| No treatment since 1931 | 0·03 | 0·33 | 4·0 |
| Heated to 60–70° C. (spring 1935) | 0·33 | Lost | 4·1 |
| Dutch white clover | | | |
| No treatment since 1931 | 0·29 | 0·30 | 3·0 |
| Heated to 60–70° C. (spring 1935) | 0·33 | Lost | 3·7 |

In the plants from the spring sowing, 1936, with red clover, there were no parasitic eelworms in the plants from any of the pots: in those from the autumn sowings, the plants from the untreated pots contained numerous *Anguillulina dipsaci*, with the typical symptoms of its attack, while those from the soil heated in 1935 were almost free, only one worm being found in the material sent to Goodey. In the untreated pots of alsike clover, the same eelworm was present with typical symptoms, while in those of Dutch white clover their presence was doubtful.

Generally, it seems clear that by heating the soil to a very moderate temperature, i.e. from 60 to 70° C. in the moist condition, it can again be made suitable for the growth of clover, but this improvement is temporary, and the repeated growth of the same clover on the soil rapidly brings back a condition similar to that with unheated soil. The first crop after the heating shows a very marked effect; the second less

so, and the effect progressively decreases, as shown by the size of the plants relative to those grown on unheated soil.

The effect on the prevalence of parasitic eelworms in the plants grown is wholly different. The second crop of clover after the heating, even with the big and apparently healthy plants, was full of eelworms. The third crop of clover was very much dwarfed, but Goodey was unable to find eelworms in any but one of the plants. They reappeared in the next crop very erratically, and seemed to have no connexion whatever with the dwarfing of the plants, though typical symptoms of their attack were found in some of them. The almost inevitable conclusion is that the dwarfing of the plants which, to me, is the typical sign of a clover-sick soil, seems to have no direct connexion with the attack by *Anguillulina dipsaci*, whose symptoms are quite specific and which can frequently be also present in a case of clover sickness.

This lack of connexion between clover sickness and the attack by parasitic eelworms is still more strikingly shown by the experience with crimson clover (*Trifolium incarnatum*). This crop, which was new to the land from which the present crop was taken, grew vigorously each year from 1931 to 1933, but then, in the next year (1934), refused to grow at all, and this in spite of repeated sowings. All the same, Goodey could find no *Anguillulina dipsaci* in the soil. In the winter following (1934–5) a few small plants were obtained, but there were no eelworms in them.

In the spring of 1935, the damp soil was heated to a temperature of 65–70° C. for 1–2 hours, and then again planted with crimson clover. An excellent crop was obtained, and the results with succeeding crops is shown in Table VI.

Table VI

| Date | Weight per green plant g. | Condition of plants |
|---|---|---|
| 1935 (spring | Not taken | Excellent crop, ripening normally |
| 1935 (autumn) | 6·15 | Very good crop |
| 1936 (spring) | 10·8 (1 plant only) | One plant healthy and well grown |
| 1936 (autumn) | 10·3 | Several plants died. Remaining plants good |
| 1937 (spring) | No plants | No growth obtained in spite of repeated sowing |
| 1937 (autumn) | Small plants | Poor dwarfed plants |

Now, with this crop, Goodey has never been able to find any *Anguillulina dipsaci* either in the healthy or in the unhealthy plants, or in the soil on which they are grown. Yet the plants have tended to become dwarfed and to refuse to grow, and the soil has again been made

healthy for the crimson clover by simple heating, in the moist condition, *to a temperature of 60–70° C.*

It seems that we are entitled to conclude that while parasitic eelworms are frequently found in the dwarfed plants grown on clover-sick soil, and, in fact, the typical results of their attack are frequently seen in them, yet they do not represent the primary cause of the dwarfed condition, which is what farmers generally know as clover sickness.

### (4) *The effect of large quantities of farmyard manure*

One of Gilbert's most interesting observations seventy years ago was that clover continued to produce normal growth very much longer on rich garden soil than on ordinary fertile field soil. This observation has been repeated many times since his day, and at Rothamsted it has been possible to maintain some sort of a crop of red clover on Gilbert's original piece of garden land to the present day. Hall (1917) records that the yield of the clover on this land during the first sixty years of the experiment was as follows:

| | | |
|---|---|---|
| 25 years, 1854–78. | Average yield (per acre), 7664 lb. clover hay. | |
| 25 years, 1879–1903. | ,, ,, 3924 | ,, |
| 10 years, 1904–13. | ,, ,, 3333 | ,, |

Towards the end of this period increasing difficulty was found in maintaining a crop of red clover, and it became infected by *Sclerotinia trifoliorum*. But even then, an occasional crop of excellent character was obtained. Nevertheless, generally speaking, it became increasingly difficult to maintain or produce a plant of clover on this land, and the clover that did grow was very small and feeble. Since the date of Hall's figures given above, the following crops have been obtained:

| | | |
|---|---|---|
| 10 years, 1914–23. | Average yield (per acre), 1542 lb. clover hay | |
| 1934. | ,, ,, 68 | ,, |
| 1935. | ,, ,, 33 | ,, |
| 1936. | ,, ,, 823 | ,, |
| 1937. | ,, ,, 1407 | ,, |

In view of the experience in this case, it seemed possible that if the garden land conditions could be more or less reproduced, it might be possible to bring back soil which was intensely clover sick to a condition in which it was again capable of growing a healthy and normal clover plant. Now the chief difference between a good garden soil and an ordinary field soil is that the former, while kept in a considerably better tilth

than is possible in the field, is also treated far more heavily with organic manures and especially with farmyard manure.

It is very interesting, in this connexion, to recall the rather cautious but very suggestive notes of Lawes & Gilbert in 1860. At that time they wrote as follows:

> How then are we to account for the fact that whilst, under the conditions described, the clover plant would not be able to grow healthily in the experimental field, we have been able to cut fourteen crops from seed sown six years ago in a garden only a few hundred yards distant? Are we to suppose, simply, that the ultimate constituents required by the clover were more abundantly available to the plant in the garden soil? or is it that they there existed in different states of combination?...If we were to suppose that some plants (clover for example) required for healthy growth a certain proportion of their food to be presented to them in the form of such carbon compounds, more complex than carbonic acid, and perhaps combined with ammonia, we should then the more easily comprehend why it should be necessary for a certain period of time to intervene before again cultivating certain crops on the same land: for we could easily understand that this might be requisite for the gradual formation and accumulation of a sufficient amount of the compounds in question.

Against this, Lawes & Gilbert's experiments with dressings up to 15 tons of farmyard manure per acre (1860) did not seem to indicate that such additions to ordinary field soil, either alone or in combination with large dressings of lime, materially delayed the time before the reduction of yield set in, which was the evidence of a clover-sick soil. It seemed likely that if there was any specific effect of farmyard manure in preventing or curing clover sickness in soil, it would only be effective in cases where the amount is very large, and where the soil is brought by this means more into the condition of a garden soil.

Experiments were, therefore, undertaken with soil which had proved its incapacity to grow the respective clovers in the previous season even when well treated with calcium carbonate and potassium phosphate (see p. 443). These were treated with 135 g. per pot of dried farmyard manure, containing 2·22 % nitrogen, mixed with the top 6 in. of the soil. This is equal to 9·5 % of wet farmyard manure on the soil to 6 in. deep. The pots were then sown with the respective clovers. From the beginning the plants grew well, and in all cases they gave an excellent crop. The subsequent history of these pots is shown in Table VII. It may be noted that there was no drainage from these pots.

It is quite clear from these figures that farmyard manure added in a large enough quantity is able to do away with, for the time being, the intense clover sickness which exists in this soil, and that the benefit is still capable of being detected for at least two years after its addition.

The amount of farmyard manure added in this case, however, is, as already stated, very large, and is obviously an impossible quantity to use practically, though it brings the rather poor field soil to a condition which approaches that in a highly cultivated garden soil.

Table VII

| Clover and date | Weight per plant g. | Notes on crop |
|---|---|---|
| Broad red clover | | |
| *1936 spring crop* | | |
| No manure | No crop | — |
| Manured as above | 5·0 | No eelworms in plants |
| 1936 autumn crop | | |
| No manure | 0·9 | — |
| Manured as above | 11·6 | — |
| 1937 spring crop | | |
| No manure | 0·7 | Eelworms plentiful |
| Manured as above | 7·9 | No eelworms |
| 1937 autumn crop | | |
| No manure | 2·3 | — |
| Manured as above | 11·4 | — |
| Alsike clover | | |
| 1936 spring crop | | |
| No manure | 0·005 | — |
| Manured as above | 2·75 | No eelworms in plants |
| 1936 autumn crop | | |
| No manure | 0·03 | — |
| Manured as above | 4·7 | — |
| 1937 spring crop | | |
| No treatment | 0·3 | Eelworms present |
| Manured as above | 3·9 | No eelworms present |
| 1937 autumn crop | | |
| No treatment | 4·0 | — |
| Manured as above | 11·0 | — |
| Dutch white clover | | |
| 1936 spring crop | | |
| No treatment | 0·05 | No eelworms present |
| Manured as above | 2·40 | No eelworms present |
| 1936 autumn crop | | |
| No treatment | 0·3 | — |
| Manured as above | 9·4 | — |
| 1937 spring crop | | |
| No treatment | 0·3 | Few, if any, eelworms |
| Manured as above | 2·6 | Few eelworms |
| 1937 autumn crop | | |
| No treatment | 3·0 | — |
| Manured as above | 9·1 | — |

The next question, however, is to see whether a similar effect can be produced with a more reasonable quantity, and so in the autumn of 1936 farmyard manure containing a fifth of the amount of nitrogen previously used (or 1 g. per pot) was added to another set of pots. This involved the addition of 45 g. of dried farmyard manure per pot, and was equal to, approximately, 3 % of the soil in farmyard manure in the

condition in which it would be usually applied. Since that time two crops have been taken, one reaped in spring 1937 and one in the autumn of the same year. The results are shown in Table VIII.

Table VIII

| Clover and date | Weight per plant g. | Notes on crop |
|---|---|---|
| Broad red clover | | |
| 1937 spring crop | | |
| No treatment | 0·7 | Eelworms plentiful |
| Manured as above | 8·0 | Eelworms plentiful |
| 1937 autumn crop | | |
| No treatment | 2·3 | — |
| Manured as above | No crop | — |
| Alsike clover | | |
| 1937 spring crop | | |
| No treatment | Crop lost | — |
| Manured as above | — | — |
| 1937 autumn crop | | |
| No treatment | 4·0 | — |
| Manured as above | 9·4 | — |
| Dutch white clover | | |
| 1937 spring crop | | |
| No treatment | 0·3 | Few eelworms, if any |
| Manured as above | 5·0 | Few eelworms, if any |
| 1937 autumn crop | | |
| No treatment | 3·0 | — |
| Manured as above | 6·8 | — |

There is no douht, therefore, that the immediate result of the addition of even the smaller amount of farmyard manure now in question (though it is already a very large amount) is to increase the size of the crop, though apparently eelworms may still he found as ahundantly in the manured as in the untreated clover. The effect still persists with the second crop, though not so markedly as even in the fourth crop after the larger amount of farmyard manure, in the case of both alsike and white clover. In the case of broad red clover it was not able to prevent the complete failure of the second crop after the addition.

We are thus able to come to the very definite conclusion that in a soil which is very intensely affected with clover sickness, and which is not improved in this respect by a large dressing of mineral manures and lime, or by a large dressing of potassium nitrate (see above), we have been able to grow a normal crop of all three kinds of clover with a very large dressing of farmyard manure, and this effect has been found to continue for at least four crops, though the advantage rapidly declined.

A similar though far more transient effect was produced by the smaller dressing of farmyard manure, though my records are not complete

except with the Dutch white clover. Here the first crop after the application gave an increase of 16·7 times; the second crop gave an increase of 2·3 times. It is clear that the total effect of the smaller dressing is less marked, and that it disappears more rapidly. In fact, it appears that in the second year (the third and fourth crops) there will in all probability be little remaining effect, and the sickness will have become as virulent as ever.

### Conclusions

This is as far as the present observations and experiments have gone. It has become clear that, in the case of the particular soil in question, no manuring with lime, phosphates or potash or with easily available nitrogen in the form of potassium nitrate have any considerable effect on the capacity of the soil to prevent the growth of a crop of any of the clovers that have been used for experiment. Two methods have, however, been effective in giving a temporary relief. The first is the heating of the moist soil to a temperature of 60–70° C. for 1–2 hours. The effect of this treatment, however, quickly passes off, and the soil becomes as virulent as ever. No attempt at reinfecting such a heated soil with plants from a pot which is virulently clover sick has been effective in preventing the success of the heating. The other treatment which has given a positive result is the application of a very large dressing of farmyard manure. This has been at first quite effective, but the quantity required is not such as could be used in practice, nor can its action be taken as one of ordinary manuring. Where nearly 10 % of the weight of the soil was added in the form of wet farmyard manure, the result was detectable after four crops of clover had been grown; where 3 % of farmyard manure (wet) was added, the effect in the one case where a comparison is obtainable was very rapidly passing away after the second crop.

While the experiments described seem to make it quite clear that, though the reduction in size of the clover plant (which I have defined as essentially what is considered as clover sickness in the agricultural sense) is often accompanied, in the present case, by the attack of the eelworm *Anguillulina dipsaci*, yet it can be distinguished from the latter and can, indeed, exist without it. It is quite possible that results in other cases of clover sickness may be different, but all my results so far with regard to determining the actual primary cause of the affection are negative.

It is realized that the enquiry is only at the beginning. How far the clover sickness can be reduced by merely allowing the soil to stand without a crop at all; how far the admixture of each of the clovers with grasses

or other plants may affect the attack of the disease; how far other and more practicable methods of partial sterilization of the soil can achieve the results which have been obtained by heating; and how far the peculiar and beneficial effect which has been obtained with large doses of farmyard manure can be obtained with other organic manures, will be the subject of the next stage in these experiments.

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# THE CHEMICAL EVALUATION OF PYRETHRUM FLOWERS (*CHRYSANTHEMUM CINERARIAEFOLIUM*)

## A COMPARISON OF SEVERAL METHODS

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### Introduction

During the past few years, methods have been elaborated for the determination of the pyrethrins I and II in pyrethrum flowers, based upon the reducing property of the pyrethrolone fraction of their molecules, or upon the assessment of the chrysanthemum mono- and dicarboxylic acids. Of the reduction methods, that of Gnadinger & Corl (1929) is probably the most widely used, and has been shown (Martin & Tattersfield, 1931; Gnadinger, 1936; Hartzell & Wilcoxon, 1932), to give values for the total pyrethrins in accordance with the results by the Tattersfield method, which is dependent upon the determination of the pyrethrin acids. Gnadinger & Corl (1934), however, have shown that the more recent modification of the "acid" method by Seil (1934) tends to give results in excess of those by his own method. Ripert (1934) prefers to use yet another technique in assessing the pyrethrins by their acid values, and removes any possible interfering free pyrethrin or other acids prior to analysis, while Wilcoxon (1936) determines the pyrethrin I by utilizing the reaction of the monocarboxylic acid with Denigés reagent. An alternative method of assessment of the pyrethrin II has been developed by Haller & Acree (1935), who determine the methoxyl content of a petroleum-ether extract of the flowers. It is proposed in this paper to make a comparison of the results obtained for the pyrethrins I and II by the modifications of the acid method outlined, and by the methoxyl method of Haller & Acree.

The material used consisted of the fully open flowers of *Chrysanthemum cinerariaefolium* from our experimental plots at Woburn, Beds, of samples received from Kenya, a commercial Japanese sample and of kiln- and air-dried flowers from an experimental plot at Wye, Kent. The flowers from Woburn were destalked and dried under cover at a low temperature,

All samples were in good condition, with the exception of Woburn nos. 3 and 4 which had become mildewed in drying, following the incidence of heavy rain during harvesting. They were included for this reason. The Kenya and Wye flowers were received as whole, dried heads. All were finely ground immediately before analysis. In all cases, the percentages of the pyrethrins I and II are expressed on the air-dried flowers containing approximately 10 % of moisture.

## EXPERIMENTAL

### *The occurrence of free acids in the flowers*

In the method of Tattersfield *et al.* (1929), the flowers are extracted with petroleum ether and the extract saponified after the removal of fatty material by cooling a methyl alcoholic solution. Ripert (1934) treats the petroleum-ether extract with aqueous potash in order to remove any possible interfering acids. We have adopted this principle, using decinormal potash as extractant. With our freshly harvested material, only small amounts of apparent monocarboxylic acid were removed by this treatment, the equivalent pyrethrin I values being of the order of 0·01–0·03 % of the flowers. The residual ether-soluble acid, however, appeared to influence the ultimate pyrethrin II values more markedly, being equivalent to as much as 0·05 % of the flowers.

Table I. *Free volatile, petroleum ether-soluble and non-volatile, ether-soluble acids separated by alkali from petroleum-ether extracts and expressed respectively as percentages of pyrethrin I and II in the flowers*

Tattersfield method

| Sample | Age in months and condition of the flowers | Free volatile acid calculated to pyrethrin I % | Pyrethrin I % | Free ether-soluble acid calculated to pyrethrin II % | Pyrethrin II % |
|---|---|---|---|---|---|
| Kenya no. 1 | Analysed immediately upon receipt from Kenya, good | 0·01 | *0·58* | 0·02 | *0·58* |
| 6 | ,, | 0·01 | *0·61* | 0·02 | *0·46* |
| Wye | Six, good | 0·01 | *0·66* | — | — |
| Harpenden no. 1 | ,, | 0·03 | *0·65* | 0·05 | *0·63* |
| Woburn no. 1 | ,, | 0·03 | 0·47 | 0·03 | 0·49 |
| 2 | ,, | 0·03 | 0·44 | 0·03 | 0·49 |
| 3 | mildewed | 0·02 | 0·34 | 0·05 | 0·37 |
| 4 | ,, | 0·04 | 0·41 | 0·03 | 0·37 |

Pyrethrin I and II contents determined without removal of free acids, given in italics.

The work of Ripert (1934) indicates that the immature flowers, particularly, may contain appreciable quantities of the free pyrethrin acids. In addition, there is the possibility that these may develop in

fully open flowers on storage. The suggestion by Ripert that they be removed prior to analysis is therefore a timely one, and we have incorporated this precautionary measure into all recent work upon pyrethrum.

### *The removal of possible interfering acids by precipitation with barium*

The methods of Seil and Ripert utilize the fact that the barium salts of the pyrethrin acids are soluble in water. In early work on the Tattersfield method, the effect of barium precipitation upon the pyrethrin I and II values was tested. To the aqueous solution of the potassium soaps, barium chloride solution was added, the precipitate filtered off, washed, and the clear filtrate acidified and distilled in steam in the usual way. In no case was the pyrethrin I value, calculated from the volatile acid, influenced, the figures by the normal method and by the modification employing barium treatment agreeing within experimental error. With the subsequent determination of pyrethrin II by the extraction of the water-soluble acid with successive portions of ether, the results obtained by the modification in which barium precipitation had been incorporated, were not significantly different from those given by the normal Tattersfield method (see Table II).

Table II. *The pyrethrin content of pyrethrum flowers determined with and without additional barium precipitation*

| | Tattersfield method | | | |
|---|---|---|---|---|
| | Volatile acid calculated to pyrethrin I % | | Water-soluble acid calculated to pyrethrin II % | |
| *Sample* | *Normal acid method* | *With barium precipitation* | *Normal acid method* | *With barium precipitation* |
| Wye | 0·66 | 0·65 | — | — |
| Harpenden no. 2 | 0·46 | 0·46 | 0·49 | 0·50 |
| 3 | 0·49 | 0·48 | 0·57 | 0·56 |
| Kenya no. 1 | 0·58 | 0·57 | 0·58 | 0·60 |

*Free acids not removed.*

### *A comparison of the Seil and Tattersfield methods*

In 1934 Seil published a modification of the acid method in which barium precipitation was incorporated. There was, however, no preliminary removal of free acids from the petroleum-ether extract of the flowers. We have carried out comparative analyses by the Seil and Tattersfield methods, with and without preliminary removal of free acids. In all cases the extraction of the dicarboxylic acid in the Tattersfield method was effected by successive extractions with ether in a separating funnel. The agreements in the results were found to be good, particularly

in the case of pyrethrin I. The Seil determination of the pyrethrin I is more rapid than in the Tattersfield method, but that of pyrethrin II appears to be more tedious, and there is seen a tendency for the values for pyrethrin II by the Seil method to be slightly greater than those by the Tattersfield method (Table IV).

### *The Ripert method*

The samples used in the comparison of the Seil and Tattersfield methods were subjected to analysis by the method of Ripert. In this the total acids are first titrated, the volatile fraction distilled off, using superheated steam, and the pyrethrin II acid determined by difference. In all cases the free acids occurring in petroleum-ether extracts of the flowers were removed by preliminary potash treatment.

Ripert recommends the use of superheated steam, and the collection of two portions of 100 c.c. each of distillate. In the tests carried out on the Kenya no. 1 sample, steam at 100° C. and four portions of distillate of 100 c.c. each were collected. By extraction of the first two fractions the pyrethrin I figure recorded was 0·62 %, and the corresponding pyrethrin II 0·71 % of the flowers. On extraction of the third and fourth distillate fractions, the pyrethrin I figure rose to 0·67 %, the pyrethrin II then being also 0·67 %. It wonld thus appear necessary, if superheated steam is not used, to collect four fractions of 100 c.c. each of distillate. In all subsequent tests, steam at 180–200° C. was used, and two fractions of 100 c.c. each collected. The results of the tests on the Kenya no. 1 sample are given in Table III.

Table III. *Analysis of Kenya flowers by the methods of Ripert, Tattersfield and Seil*

| Treatment | % of flowers: Pyrethrin I | Pyrethrin II |
|---|---|---|
| Ripert method. Petroleum-ether extraction, free acids not removed, steam at 100° C., four fractions of 100 c.c. each collected. | | |
| Extraction of first and second fractions | 0·62 | 0·71 |
| Extraction of total distillate | 0·67 | 0·67 |
| Ripert method. Petroleum-ether extraction, free acids removed, extract acidified and distilled in steam, volatile and ether soluble acids separated as in Tattersfield method | 0·01 | 0·03 |
| Analysis continued on petroleum-ether solution using superheated steam | 0·64 | 0·64 |
| Tattersfield method. Petroleum-ether extraction, free acids not removed | 0·58 | 0·58 |
| Tattersfield method. Free acids not removed, barium precipitation incorporated | 0·57 | 0·60 |
| Seil method. Free acids not removed | 0·59 | 0·57 |

*The Haller & Acree method for pyrethrin II*

This was carried out, using petroleum-ether extraction of the flowers in a Soxhlet apparatus. The extract, after transference to the methoxyl apparatus with chloroform, was heated in a boiling-water bath under reduced pressure to remove final traces of solvent. The comparative results by the Tattersfield, Seil, Ripert and Haller & Acree methods are given in Table IV.

Table IV. *Comparison of results by the Tattersfield, Seil, Ripert and Haller & Acree methods*

| Sample | Tattersfield | Seil | Ripert | Haller & Acree |
|---|---|---|---|---|
| | Pyrethrin I % of flowers | | | |
| Woburn no. 1 | 0·47 | 0·47 | 0·52 | — |
| 2 | 0·44 | 0·42 | 0·49 | — |
| 3 | 0·34 | 0·36 | 0·37 | — |
| 4 | 0·41 | 0·40 | 0·41 | — |
| Kenya no. 1 | *0·58* | *0·59* | 0·64 | — |
| 2 | *0·64* | *0·65* | 0·68 | — |
| 3 | *0·67* | *0·67* | 0·70 | — |
| 4 | *0·66* | *0·63* | 0·66 | — |
| 5 | *0·56* | *0·55* | 0·54 | — |
| | Pyrethrin II % of flowers | | | |
| Woburn no. 1 | 0·49 | 0·54 | 0·70 | *0·56* |
| 2 | 0·49 | 0·57 | 0·69 | *0·54* |
| 3 | 0·37 | 0·39 | 0·47 | *0·40* |
| 4 | 0·37 | 0·41 | 0·44 | *0·45* |
| Kenya no. 1 | *0·58* | *0·57* | 0·64 | *0·60* |
| 2 | *0·46* | *0·50* | 0·65 | *0·64* |
| 3 | *0·52* | *0·52* | 0·68 | *0·59* |
| 4 | *0·52* | *0·50* | 0·57 | *0·50* |
| 5 | *0·52* | *0·53* | 0·52 | *0·46* |

Figures in italics = free acids not removed before analysis.

Further analyses using the Haller & Acree method for the determination of pyrethrin II were carried out upon six samples of flowers received from Kenya that had been dried at different temperatures. The samples were analysed by the Seil method independently by Messrs Salamon and Seaber, of London, who kindly permit us to give their values for pyrethrin II for comparison. The Haller tests were carried out immediately after the determinations by the Seil method.

Later, work was carried out upon flowers grown at Wye, Kent, to test the possible effect upon the pyrethrin content of drying with a current of air at relatively high temperatures. Analyses for pyrethrin II were carried out by the Seil and Haller methods. A full description of the experiment has been published elsewhere (Jary *et al.* 1937). The com-

parative results obtained with the Kenya and Wye samples are combined in Table V.

Table V. *The determination of pyrethrin II in Kenya and Wye flowers by the methods of Seil and Haller & Acree*

| Sample | | Pyrethrin II % of the flowers: Seil | Pyrethrin II % of the flowers: Haller & Acree |
|---|---|---|---|
| Kenya. | Sun dried | 0·58 | *0·61* |
| | Dried at 38° C. | 0·71 | *0·78* |
| | ,, 43° C. | 0·63 | *0·78* |
| | ,, 49° C. | 0·66 | *0·66* |
| | ,, 54° C. | 0·72 | *0·76* |
| | ,, 65–76° C. | 0·75 | *0·78* |
| Wye no. 1. | Air dried | 0·70 | *0·66* |
| 2. | ,, | 0·73 | *0·64* |
| 3. | ,, | 0·69 | *0·62* |
| 4. | ,, | 0·67 | *0·61* |
| 5. | ,, | 0·73 | *0·68* |
| Wye no. 1. | Kiln dried 60° C. 6¼ hours | 0·69 | *0·81* |
| 2. | ,, 45° C. 21 ,, | 0·67 | *0·78* |
| 3. | ,, 75° C. 3½ ,, | 0·62 | *0·74* |
| 4. | ,, 52° C. 10 ,, | 0·68 | *0·75* |
| 5. | ,, 68° C. 5¾ ,, | 0·58 | *0·72* |

Figures in italics = free acids not removed before analysis.

While the figures in no way indicate the possible effect upon the pyrethrin content of the Kenya flowers of drying at relatively high temperatures owing to the absence of sun-dried controls for each temperature used, they demonstrate that for artificially dried flowers, the values for pyrethrin II by the Haller method tend to be in excess of those by the Seil method.

In the Wye samples dried under cover at air temperatures, the pyrethrin II figures by the Haller method are, without exception, somewhat lower than those by the Seil method, but for the kiln-dried material higher values are obtained in all cases. We are unable, at the moment, to explain this peculiar tendency of the Haller method to give high values for the artificially dried flowers.

### *The Wilcoxon method for pyrethrin I*

Wilcoxon (1936), in an examination of the Seil method, concludes that either the monocarboxylic acid is not entirely volatile in steam, or there is an appreciable loss during distillation, partly compensated by the presence of volatile, petroleum ether-soluble acid material other than the pyrethrin I acid. He proposes as an alternative to the determination of the monocarboxylic acid by distillation, the quantitative

reduction of Denigés acid mercuric sulphate reagent, the preliminary treatment being carried out as in the Seil method. Wilcoxon states that the sample taken for the analysis should contain 50–70 mg. pyrethrin I. In all our initial tests, however, the reduction was carried out using an extract equivalent to 10 g. of flowers, and as the samples used ranged from poor to rich flowers, the limits of concentration given by Wilcoxon were not strictly observed. In both methods the free acids were first removed from the petroleum-ether extracts of the flowers. The results are given in Table VI.

Table VI. *Comparison of the Seil and Wilcoxon methods for pyrethrin I*

| Sample | Pyrethrin I % of the flowers: Seil | Wilcoxon |
|---|---|---|
| Woburn no. 5 | 0·27 | 0·27 |
| 6 | 0·45 | 0·50 |
| 7 | 0·36 | 0·42 |
| 8 | 0·31 | 0·33 |
| 9 | 0·45 | 0·53 |
| German strain, Harpenden grown | 0·34 | 0·40 |
| Swiss no. 1 strain, ,, | 0·42 | 0·46 |
| 2 strain, ,, | 0·37 | 0·41 |
| Wye no. 1 | 0·64 | 0·80 |
| 3 | 0·70 | 0·83 |
| 4 | 0·72 | 0·90 |
| 5 | 0·79 | 1·01 |
| Wye no. 1. Kiln dried 60° C. | 0·68 | 0·78 |
| 3. ,, 75° C. | 0·63 | 0·71 |
| 4. ,, 52° C. | 0·71 | 0·86 |
| 5. ,, 68° C. | 0·69 | 0·91 |

The values for the pyrethrin I by the Wilcoxon method are, in all cases except the low-quality Woburn no. 5 sample, in excess of those by the Seil method. As the richness of the flowers increases, the greater appears to become the percentage excess of the Wilcoxon over the Seil value, culminating in a divergence of the order of 30 % in the case of the very rich Wye no. 5 sample.

Wilcoxon, however, in the published description of his method, suggests limits of concentration of pyrethrin I between which the determination should be made. The rigid observance of these would imply a preliminary analysis of a sample of flowers before the weight necessary to give a suitable concentration of pyrethrin I in the final extract could be calculated, a matter of some inconvenience. Tests were therefore carried out to determine the relationship between widely varying amounts of monocarboxylic acid taking part in the reduction, and the volumes of

iodate solution used. The observance over a wide range of the relationship 1 c.c. 0·01 *M* iodate = 4·4 mg. pyrethrin I, would permit the ready application of the method to samples of all degrees of quality.

In the first trial to test this relationship, 50 g. of Woburn flowers, three to four months old, were extracted with petroleum ether. The free acids were removed with *N* sodium hydroxide, the solvent removed, and the resin refluxed with 50 c.c. of 0·5 *N* alcoholic soda. The alcohol was evaporated by gentle warming under reduced pressure, the residue dissolved in water and precipitated with 40 c.c. of 10 % barium chloride with the addition of "celite" filter aid. The precipitated barium salts were filtered off, well washed with water, and the filtrate and washings made up to 500 c.c. The Seil distillation was carried out on 100 c.c. of the solution after dilution and acidification with 1 c.c. of concentrated sulphuric acid, the volatile and petroleum ether-soluble acid being equivalent to 0·58 % of pyrethrin I in the flowers. Aliquots of 100, 75, 50 and 25 c.c. of the solution, made up in each case to 200 c.c., were then subjected to the Wilcoxon process. The pyrethrin I values were respectively 0·76, 0·67, 0·63 and 0·64 % of the flowers.

In later tests, 50 g. of Woburn flowers were extracted with petroleum ether, and the preliminary treatment carried out as before. After precipitation with barium, the filtrate and washings (volume 350 c.c. in all) were acidified with sulphuric acid, and the monocarboxylic acid extracted with two portions of 100 c.c. each of petroleum ether. The petroleum-ether extracts were washed, combined and extracted with 0·2 *N* sodium hydroxide. Gentle rotation avoided the formation of an emulsion. The slightly alkaline water layer was run off, and with the washings of the petroleum ether, made up to 50 c.c. On submitting 10 c.c. of this solution, diluted to 25 c.c. and acidified with excess of sulphuric acid, to the semimicro distillation as used in the Tattersfield method, a figure of 0·57 % of pyrethrin I in the flowers resulted. Further aliquots of 10·0, 7·5, 5·0 and 2·5 c.c., diluted in each case to 12·0 c.c., gave respectively 0·75, 0·75, 0·63 and 0·64 % of pyrethrin I by the Wilcoxon method.

In a further comparison, 10 c.c. of a 50 c.c. solution containing the sodium monocarboxylate from 50 g. of another sample of flowers were submitted to the Seil process, but in this case the diluted solution was acidified with 5 c.c. of 0·1 *N* sulphuric acid. The pyrethrin I value was 0·63 % of the flowers. Aliquots of 10·0, 7·5, 5·0 and 2·5 c.c. gave by the Wilcoxon method 0·73, 0·68, 0·61 and 0·62 % respectively.

The relationship between the amount of the sodium monocarboxylate solution taken and the c.c. of iodate required is, therefore, not a linear

one, the greatest discrepancies between the Seil and Wilcoxon values occurring with the more concentrated solutions. When only a slight excess of acid was present in the Seil distillation, the value for pyrethrin I obtained agreed with the lower Wilcoxon figures. The Wilcoxon values given by the aliquot containing the equivalent of 10 g. of the flowers was still, however, in excess of the Seil value.

### *The effect of sulphuric acid in the distillation upon the Seil value for pyrethrin I*

Wilcoxon has indicated that the chrysanthemum monocarboxylic acid is partially destroyed by distillation in the presence of sulphuric acid, and the analyses described seem to confirm this suggestion. Tests were, therefore, carried out at this stage to determine, in the Seil method, the effect of varying concentrations of acid present in the distillation upon the amounts of volatile acid yielded.

Finely ground Woburn flowers (50 g.) were extracted with petroleum ether, the free acids were removed, and the preliminary treatment carried out as suggested by Seil, with the exception that the alcohol after saponification was removed at a low temperature under reduced pressure. The freed pyrethrin I acid in the final solution was taken up with petroleum ether, recovered as the sodium salt, and the solution adjusted to 50 c.c. Fractions of 10 and 5 c.c. gave respectively 0·69 and 0·62 % of pyrethrin I in the flowers by the Wilcoxon test. Further portions of 10 c.c. each were diluted and distilled in the presence of excesses of 0·03, 0·28 and 0·70 c.c. of concentrated sulphuric acid. The volatile acid recovered, calculated to pyrethrin I, amounted to 0·64, 0·59 and 0·54 % of the flowers respectively. Thus a progressive reduction in the pyrethrin I value recorded resulted from an increasing amount of sulphuric acid present, the value obtained with the lowest concentration of acid approaching more nearly the figure obtained by the Wilcoxon method. The values for pyrethrin I determined by the Seil method, as published, are thus low on account of the excess acid present in the distillation, and some modification is needed to reduce the loss due to this circumstance.

Further experiments were therefore carried out, using samples of Japanese and Kenya flowers, in which only slight excesses of sulphuric acid were present in the distillation. Portions of 35 g. of the finely ground flowers were extracted with petroleum ether for a minimum period of 20 hours, the free acids were removed and the pyrethrins saponified with 0·5 *N* ethyl alcoholic soda. The alcohol was removed

under reduced pressure, the residues dissolved in water, precipitated with barium chloride, and the filtrates, with the washings of the precipitates made up to 350 c.c. Aliquots of 100 c.c. each were then used as shown in Table VII.

Table VII. *Pyrethrin I % of the flowers, calculated from the volatile, petroleum ether-soluble acid*

| | Japanese | Kenya |
|---|---|---|
| (*a*) Solution acidified with 1 c.c. of concentrated sulphuric acid | 0·33 | 0·60 |
| (*b*) Solution neutralized to litmus with *N* sulphuric acid, 1 c.c. added in excess, Seil distillation | 0·39 | 0·68 |
| (*c*) Solution subjected to Wilcoxon method | 0·39 | 0·70 |

In order to determine whether distillation with a slight excess of 1 c.c. of *N* sulphuric acid causes a loss in the monocarboxylic acid, a separate test was carried out, using the rich Kenya sample. 25 g. of the flowers were extracted, and the extract treated as before, the filtrate after the barium precipitation being made up to 250 c.c. Of this, 100 c.c. were neutralized to litmus with *N* sulphuric acid, and then distilled after the addition of 1 c.c. in excess. The volatile acid extracted by petroleum ether required 9·9 c.c. of 0·02 *N* alkali. A second 100 c.c. was acidified, and extracted twice with 50 c.c. portions of petroleum ether, each extract being washed free from sulphuric acid with small amounts of water. The combined petroleum-ether solution was distilled gently from 200 c.c. of water, and a Seil distillation then carried out. The petroleum ether was used to make the first extraction of the distillate. The petroleum ether-soluble acid in the distillate was also equivalent to 9·9 c.c. of 0·02 *N* alkali. Thus there would appear to be little or no loss from the use of 1 c.c. of *N* acid in excess in the distillation.

We have shown (Martin & Potter, 1937) that a colourless petroleum-ether extract of pyrethrum flowers may be obtained by intimately mixing decolorizing charcoal with the finely ground plant material prior to extraction. A colourless extract of the Kenya sample was so obtained and submitted to the modified Seil process. The pyrethrin I content of the flowers determined by the analysis of the colourless extract was the same as that determined on a normal extract made without the use of charcoal.

*The solvent to be used for the initial extraction of the flowers*

Ripert (1934, 1936) has pointed out the possibility of the incomplete extraction of the pyrethrins by petroleum ether, which he ascribes to the mechanical holding up of the poisons by a film of oxyacids, soluble only with difficulty in petroleum ether. He states that extraction with ethyl ether, either directly or subsequent to petroleum ether, will bring about a more complete separation, but Gnadinger (1936) has indicated the danger of the inclusion in the extract of changed pyrethrins by this procedure.

In all our analyses we have used petroleum ether as extractant, but have carried the time of extraction beyond the 8 hours normally recommended for the process. Rapidly refluxing Soxhlets were allowed to run for at least 20 hours, with a soaking period overnight. Following such extraction of flowers and an exposed talc-pyrethrum dust we have found only small amounts of pyrethrin I to be recovered subsequently by ether when a barium precipitation has been incorporated. Large additional quantities of apparent pyrethrin II have, however, resulted from ether extraction subsequent to petroleum ether, even when barium precipitation has been used to separate possible interfering acids. The results obtained in the Seil and Tattersfield methods using petroleum ether and ether extraction, with and without the incorporation of barium precipitation in the latter method, are summarized in Table VIII.

Table VIII. *Analysis of talc-pyrethrum dust containing approximately* 0·5 % *of pyrethrin I, exposed for* 80 *hours to* 1000 *watt lamp*

| | Pyrethrin I | Pyrethrin II |
|---|---|---|
| Tattersfield method: | | |
| Before exposure, petroleum ether | 0·52 | 0·51 |
| After exposure, petroleum ether | 0·02 | 0·15 |
| After exposure, ether after petroleum ether | 0·03 | 0·43 |

No preliminary removal of free acids.

*Analysis of Wye flowers, stored for* 6 *months in tins in a cool place*

| | | |
|---|---|---|
| Petroleum ether extraction: | | |
| Tattersfield method | 0·66 | 0·56 |
| Tattersfield method with barium precipitation | 0·65 | 0·67 |
| Seil method (includes barium precipitation) | 0·66 | 0·64 |
| Ether extraction: | | |
| Tattersfield method | 0·82 | 0·83 |
| Tattersfield method with barium precipitation | 0·69 | 0·88 |
| Seil method (includes barium precipitation) | 0·72 | 0·86 |

No preliminary removal of free acids.

Further tests with other samples were carried out, using the Seil and Wilcoxon methods. Of each sample of flowers tested, 25 g. of finely

ground, but not impalpable material, were extracted thoroughly with petroleum ether, the extraction period extending for at least 20 hours. The free acids were removed with dilute soda, and the alkaline extract retained. The petroleum-ether extract was treated as in the Seil method, using double quantities of the reagents, the volume after addition of barium chloride being adjusted to 500 c.c. Filtered aliquots of 200 c.c. each were then used for the Seil and Wilcoxon determinations.

The extracts containing the sodium salts of the free acids from the four samples used were combined, barium chloride was added, the filtrate made up to a known volume, and equal aliquots taken for the determination of the apparent free pyrethrin acids by the Seil and Wilcoxon methods. In the latter, the assumption was made that 1 c.c. of 0·01 *M* iodate was equivalent to 4·4 mg. of pyrethrin I at the low concentration used.

The residues containing the dicarboxylic acid in the Seil method were tested for residual monocarboxylic acid by the Wilcoxon method, but none was found.

The material after petroleum-ether extraction was air-dried at a low temperature, and extracted for at least 20 hours with ethyl ether. The free acids in the extracts were removed, and determinations of the apparent free pyrethrin acids carried out as before. The ether solutions were subjected to the Seil preliminary treatment, and 200 c.c. aliquots used for the Seil and Wilcoxon tests. The values obtained are given in Table IX.

Table IX. *Analysis of pyrethrum flowers, using ether after petroleum-ether extraction*

| | Seil | | Wilcoxon |
|---|---|---|---|
| | Pyrethrin I | Pyrethrin II | Pyrethrin |
| Petroleum-ether extraction: | | | |
| Woburn no. 10 | 0·51 | — | 0·66 |
| 11 | 0·52 | — | 0·68 |
| 12 | 0·58 | — | 0·72 |
| Harpenden (Swiss strain) | 0·56 | — | — |
| Free acids | 0·01 | 0·02 | 0·03 |
| Ether after petroleum ether: | | | |
| Woburn no. 10 | 0·02 | 0·07 | — |
| 11 | 0·03 | 0·07 | 0·04 |
| 12 | 0·03 | 0·08 | 0·04 |
| Harpenden (Swiss strain) | 0·03 | 0·10 | — |
| Free acids | 0·01 | — | 0·01 |

Small additional amounts of pyrethrin I were recovered by the ether treatment, these being probably just outside the error of the method. Rather greater amounts of apparent pyrethrin II resulted. The effect of ether after petroleum-ether extraction was to influence markedly the

final figure for the total pyrethrins, due chiefly to the additional pyrethrin II. Only small amounts of apparent free pyrethrin acids were found in the petroleum ether or subsequent ether extracts of the flowers.

## Discussion

A general comparison of the results obtained by the Tattersfield and Seil methods, as published, shows that the values for pyrethrin I by the two methods agree closely. The suggestion by Wilcoxon, however, that distillation in the presence of sulphuric acid leads to a partial loss of the monocarboxylic acid, is supported by the experimental data recorded, and the necessity for a modification in the Seil process to ensure the more complete recovery of the acid is shown. This has been effected by distilling directly the monocarboxylic acid extracted by petroleum ether from the final acidified solution of the barium salts, or more simply, by neutralizing to litmus, and adding 1 c.c. of normal acid in excess before distillation. The pyrethrin I contents of the flowers determined by these means approached more nearly the values given by the Wilcoxon method. Wilcoxon states, however, that the volatile and petroleum ether-soluble acid yielded in the Seil method contains a proportion of acid other than the monocarboxylic acid. The closer agreement with the Wilcoxon values, by this hypothesis, would appear to be dependent upon a chance compensation of error.

It is likely that the figures for pyrethrin I recorded by the Tattersfield method have also been influenced by partial loss due to the action of the sulphuric acid present during distillation. In their early work on pyrethrum, Tattersfield (1929) and his co-workers found that on distillation of a quantity of the pure monocarboxylic acid equivalent to 10 g. of flowers, 98 % could be recovered from the first 40 c.c. of distillate, further fractions showing only slight acidity. In this quantitative recovery, however, no sulphuric acid was present.

In the determination of the pyrethrin II by the two methods, the Seil method tends to give slightly higher results than the Tattersfield method. The discrepancies in the total pyrethrins noted by Gnadinger between the Seil and his own method are probably largely due to these relatively higher values for pyrethrin II.

The Ripert method, in our hands, has given values for both pyrethrin I and II rather higher than the results by the Tattersfield and Seil methods. Ripert extracts a larger quantity of flowers and recommends superheated steam for the separation of the volatile acid. The collection of a greater volume of distillate than that suggested by Ripert is, however, shown to

be necessary if superheated steam is not used. Wilcoxon states that the sources of error inherent in the Seil method are applicable also to the Ripert method. Ripert (1936), in a comparison of the Seil method with his own, finds that the pyrethrin I values by his method tend to be somewhat higher than those by the Seil method, and this tendency is seen in our own results.

In our work, the figures for pyrethrin II determined by the Haller & Acree method have seemed to depend upon the origin of and the method of drying the flowers. In some cases they have been below those given by the Seil method, and sometimes above, but in nearly all cases where artificial drying has been employed, they have been on a distinctly higher level.

The preliminary separation of acids occurring freely in the petroleum-ether extracts of the flowers, suggested by Ripert, is of value as a precautionary measure, but with our own samples we have never found more than small amounts of free volatile and petroleum ether-soluble acid, although the figure for pyrethrin II may be more markedly influenced by the presence of free non-volatile, ether-soluble acid, giving a water-soluble barium salt. The combined effect of the free acids in our samples has been to influence particularly the final values for the total pyrethrins, due in a large measure to the preponderating free non-volatile acid, calculated as pyrethrin II. We have had little experience of commercial flowers, and are unaware of the extent to which interfering free acids may be present in such samples.

In an examination of the Wilcoxon method, in which the limits of concentration suggested by him were not strictly observed, it was found that the values for pyrethrin I over a wide range of samples tended to be in excess of those given by the Seil method, but that the greatest discrepancies occurred with the richest flowers. Although the loss of the monocarboxylic acid due to the presence of excess sulphuric acid in the Seil distillation may have played a part in this effect, an examination of the Wilcoxon method showed that the relationship between the mg. of monocarboxylic acid present and the c.c. of iodate solution recorded was not a strictly linear one. Aliquots were taken from a solution containing the sodium salt of the monocarboxylic acid, and diluted to the volume suggested by Wilcoxon in carrying out the reduction. The solutions with the higher concentrations of the monocarboxylic acid required, in proportion to the acid present, greater volumes of iodate solution than did those with the lower concentrations. The closer agreements between the Seil and Wilcoxon values with the poorer samples of flowers may

be thus partially explained. It is of obvious value to have alternative means to the acid methods of determining the pyrethrins, but it would appear that, in the Wilcoxon process, further work is required to account for the non-linearity of the relationship between the iodate required and the monocarboxylic acid present as the latter increases in amount.[1]

The methods used have, in nearly all cases, placed the samples examined in the same order of pyrethrin content, but there has, in some of the tests, been poor agreement in the absolute values of the pyrethrins I and II recorded. Until a standard method of analysis has been adopted, it would appear necessary to stipulate by what method analyses have been carried out if absolute values for the pyrethrins are required.

The effect of the solvent used in extraction upon the separation of the pyrethrins from the flowers has been examined in a preliminary way. Ripert (1936) has stated that a chloroform extract of flowers made after petroleum-ether extraction was highly toxic to house flies. Gnadinger (1936) has pointed out the possibility of the extraction of changed pyrethrins by the use of ether, and these would probably show lower toxicities than those of the unaltered pyrethrins.

Tattersfield, in unpublished work in 1927, found only slight toxic action to *Aphis rumicis* of ether and alcoholic extracts made after petroleum-ether extraction of Harpenden-grown flowers. The petroleum-ether extract was completely toxic at 0·01 % concentration, whereas the subsequent ether and alcoholic extracts showed little or no toxic action at concentrations of 0·25 %. Petroleum ether was therefore suggested for extraction purposes. Tattersfield (1932, p. 413) also found that while a petroleum-ether extract of pyrethrum flowers gave almost a complete kill of *Aphis rumicis* at a concentration equivalent to 0·0005 % of pyrethrin I, a subsequent alcoholic extract showed only a slight effect at a concentration equivalent to 0·002 % of apparent pyrethrin I.

It is evident from our experiments that Soxhlet extraction with petroleum ether for a minimum of 20 hours suffices for the separation of nearly the whole of the pyrethrin I. Whether toxic material is left behind either in the form of adsorbed unaltered pyrethrins, or of altered pyrethrins still possessing insecticidal properties, can only be determined by carefully controlled toxicity trials. Before petroleum ether be replaced by another solvent, the insecticidal activity of the excess material extracted should be shown to be commensurate with its apparent pyrethrin content.

[1] This paper was sent to the press before the results of an examination of the Wilcoxon method by D. A. Holaday (1938, *Industr. Engng Chem.* Anal. ed. **10**, 5) came to our notice.

SUMMARY

1. Comparative analyses of pyrethrum flowers have been carried out by the methods of Tattersfield, Seil, Ripert, Haller & Acree and Wilcoxon.

2. The methods were of value in indicating the relative richness in pyrethrins of the samples tested, but discrepancies were seen in the absolute values of the pyrethrins I and II recorded. Under present conditions and until a standard method of analysis is agreed upon, it would appear requisite to state the method employed in the evaluation of the flowers.

3. The Wilcoxon method has given higher figures for the pyrethrin I content than the Seil method. The degree of divergence between the results depended upon the richness of the flowers, and upon the excess of acid used in distilling the volatile acid in the Seil method. The relationship between the amount of the pyrethrin I acid present and the titration recorded in the Wilcoxon method was not a linear one.

4. The question of the solvent to be used for the initial extraction of the flowers has been briefly discussed.


*The determinations of pyrethrin II by the Haller & Acree method* were carried out by Dr F. Tattersfield. I thank him for this and also for helpful criticism and advice. I wish also to thank Mr J. R. Williams for assistance in the analytical work.

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# RELATION OF EAR SURVIVAL TO THE NITROGEN CONTENT OF CERTAIN VARIETIES OF BARLEY

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WITH A STATISTICAL STUDY

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(With Nineteen Text-figures)

## Introduction

The two predominant varieties of malting barley in cultivation in the British Isles, namely Plumage-Archer and Spratt-Archer, are both characterized by high grain-yielding capacity and by a total nitrogen content in the grain which is relatively lower than that of the varieties they have replaced in general use in recent years. The agricultural value of both varieties is further enhanced by the possession of straw highly resistant to lodging.

It has long been realized that the nitrogen content of barley grain is largely influenced by the character of the soil on which the crop is grown, on the weather conditions during growth, and inevitably on the combination of these two influences. But these are superimposed on the influence of the variety itself, and it is primarily with this varietal attribute that the plant breeder is concerned when choosing parents for crossing and, later, in selecting individual lines within hybrid progenies for further propagation. The differential response of varieties to their environment is a separate study.

The results of investigations prosecuted in this country and elsewhere all emphasize the strongly individual character of varieties in respect of the nitrogen content of the grain. There are indications, nevertheless, that what may be regarded as the basic genetic constitution of a variety in respect of the nitrogen content must be viewed in relation to other plant characters.

It has been shown by Engledow & Wadham (1924) from the results of various chess-board trials that there is a high correlation between the yield of grain and the number of ears per unit area, and, since in the investigations they described there was a definite initial number of plants

per plot, the correlation between yield of grain per plot and the average number of ears per plant, that is, the average tiller survival, was also high.

If the yield of grain in the case of barley in this country is so definitely associated with a high number of surviving ears, and the general trend in recent years is towards lower nitrogen in the grain, the possible relationship of high ear survival and low total nitrogen becomes a matter of considerable importance from the breeding point of view.

The objective of the breeder is a combination of yield and quality, and breeding procedure would be greatly facilitated if, in selecting forms with high ear survival rates, there was a concomitant assurance of securing low total nitrogen contents. But the production of barley suitable for malting is only one, although admittedly an important, aspect of barley breeding, and changes in economic conditions may result in a demand for feeding barleys, that is barleys of high grain-yielding capacity and preferably with a high protein content.

Russell & Bishop (1933) in summarizing the results of a series of barley variety trials and malting tests carried out in England have drawn attention to the lower nitrogen content of the high grain-yielding varieties under their review, and to the lack of any indication of the possibility of producing high grain-yielding varieties with high nitrogen contents.

From the breeding point of view these conclusions seem to imply the impossibility of obtaining purely feeding barleys of the general character designated above as distinct from malting barleys. There are, however, certain relevant considerations here which deserve attention; the first is that up to the present no extensive study has been made of the high nitrogen segregates of hybrid progenies comparable with that devoted to low nitrogen forms. Secondly, there are in existence varieties such as Archer-Goldthorpe 4/5/1 with a relatively low grain-yielding capacity which are nevertheless low nitrogen barleys (Russell & Bishop, 1933; Hunter, 1926); this suggests at least the possibility of the existence of forms at the alternative end of the scale.

The results of a series of investigations carried out by Barbacki (1933) are instructive. In his experiments Barbacki attempted a genetical analysis of hybrids obtained by crossing relatively low nitrogen barleys of European origin with high nitrogen barleys of Himalayan origin. The dominance of low nitrogen was established in the $F_1$ generation, whilst forms with nitrogen contents similar to those of the parents, as well as others with intermediate and transgressive values, appeared in later generations.

A series of correlations which Barbacki established is specially

interesting; these included a positive correlation between the total nitrogen content of the grain and

> the number of ears per plant,
> the yield of straw,
> the ratio of grain to straw,
> the weight per 1000 grains and
> the flintiness of the grain,

and a negative correlation between the nitrogen content and the weight of grain per shoot. Barbacki (p. 157) emphasizes these findings in the following recommendation: "As follows from the above correlations, the breeding of brewery barleys should be conducted in the direction of obtaining less tillering forms, with an advantageous ratio of grain to straw with a maximum yield of grain per shoot, with mealy grain, and with an intermediate weight per 1000 grains. . . ."

Most of these relationships are in conformity with the characteristics generally found in plants grown under conditions of unrestricted ground space or of high soil fertility, and it will be observed that the first correlation, namely, that of nitrogen content and the number of ears per plant, appears to be a direct contradiction of the characteristics ascribed above to Plumage-Archer and Spratt-Archer. Moreover, Bell (1937) in a series of comparisons made with the three varieties, Spratt-Archer, Tschermak's, and Stadler's A, found that Spratt-Archer had the highest ear survival at harvest and produced the highest yield of grain per plot and per plant, but not the highest yield of grain per ear. Spratt-Archer nevertheless showed the lowest total nitrogen content in the grain, and a relatively much lower 1000-grain weight than the other two varieties.

It will be seen, also, from the figures that follow that in the particular varieties tested, high ear survival rate does not necessarily indicate high nitrogen content; it is indeed found concomitantly with relatively low nitrogen content and high grain yield, and thus becomes an index of both these important economic attributes.

## Experimental

Since the yield of grain in the British Isles is a reflection of the survival of a relatively large number of ears rather than of the extreme development of one ear, the various tests described below had for their immediate object the determination of the number of surviving ears per plant at harvest in relation to the yield and total nitrogen content of the grain of those ears.

Two lines of approach were chosen; the first was designed to ascertain the influence of increased tillering on the nitrogen content of the grain, and the second the influence of the abscission of tillers at various stages of plant development on the nitrogen content of the grain of the surviving shoot, $T_0$.

The first series of experiments was conducted in 1936 with Spratt-Archer, and with one small addition, which will be described later, it was repeated in 1937.

In this series, which was designed to ascertain the effect of increased ear survival on the nitrogen content of the grain, nitrate of soda was applied at the rate of 1 cwt. per acre at stages in plant development denoted by *B*, sowing; *C*, on the appearance of the first tiller above the surface of the soil; *D*, when the tillers assumed a vertical position; *E*, at flowering; *F*, at an interval of approximately 21 days after *E*. The exact dates of application, with other relevant data are shown below:

Table I. *Showing the dates of sowing and harvesting, and of the application of nitrate of soda*, 1936 *and* 1937. *The figures in parenthesis indicate the number of days between sowing and the application of the manure*

| Treatment | 1936 Spratt-Archer | 1937 Spratt-Archer | 1937 Spratt |
|---|---|---|---|
| *A* (Control) | 30/3 — | 16/4 — | 23/4 — |
| *B* | 2/4 (3) | 16/4 (0) | 23/4 (0) |
| *C* | 4/5 (35) | 18/5 (32) | 18/5 (25) |
| *D* | 6/6 (68) | 4/6 (49) | 4/6 (43) |
| *E* | 23/6 (85) | 3/7 (78) | 3/7 (71) |
| *F* | 16/7 (108) | 23/7 (98) | 23/7 (91) |
| *G* | — | {16/4 (0)<br>23/7 (98) | 23/4 (0)<br>23/7 (91) |
| Harvested | 18/8 | 18/8 | 18/8 |
| Total growth period (days) | 141 | 124 | 117 |

In the second series, tillers were removed at the following stages: *b*, as they appeared; *c*, at full tiller (number) development; *d*, when the shoots assumed a vertical position; *e*, at flowering; *f*, *ears only* of all but the main shoot removed at flowering.

After the first abscission in each treatment further tillers were removed as rapidly as they appeared.

In both series a set of control plots denominated *A* in the first and *a* in the second series was included.

The experiments were sown in the open, in plots 4 ft. square; seeding was done in drills 6 in. apart, the grains being deposited at the usual depth of $1\frac{1}{2}$ in., and at distances apart which allowed an area of 12 sq. in.

per plant. After allowing for the customary discard of 1 ft. round each plot, the final experimental area was 1 sq. yd. with a maximum survival attainment of 108 plants.

The plots were sown in a Latin square lay-out, and as the number of treatments *plus* control in 1936 was six, each treatment was replicated six times.

The investigations of the effect of the applications of nitrate of soda were repeated in 1937 with the addition of the variety Spratt in a similar but separate Latin square lay-out. The botanical and agricultural features of Spratt have been described previously (Hunter, 1926; Bell. 1937*a*). In the present investigations Spratt presents special features of interest, for it is one of the parents of Spratt-Archer and is, moreover, recognized as a variety particularly suitable to soils of high fertility such as the Fens. In the latter respect it thus offers a desirable contrast to Spratt-Archer, which is regarded as a variety capable of producing relatively higher yields of grain on less fertile classes of soil.

From the results obtained in 1936 there was evidence that an increase in ear survival could be obtained without increasing the nitrogen content of the grain. This may be interpreted as indicating the distribution of the nitrogen absorbed by the plant amongst the surviving ears, and consequently a progressively decreasing percentage of nitrogen in the grain as the number of ears increased. It was considered that the correctness of this deduction might be established by increasing the number of ears per plant, and then applying additional nitrogen at a stage when tiller development had ceased. Accordingly, in the investigations carried out in 1937 an additional set of plots was added in the case of both Spratt and Spratt-Archer, making the total number of plots with each of the two varieties 49, as compared with 36 in 1936. The *G* plots, as they were denominated, received nitrogen at sowing, as in the case of the *B* sets, and then 3 weeks after flowering as in the case of the *F* sets.

The two Latin squares, 1937, were sown in a contiguous position, and there was no reason to consider the respective pieces of ground they occupied different in fertility. Nevertheless, no varietal comparison of the two barleys is attempted in the statistical treatment of the figures which follow, although the general respective reactions of the varieties to the various treatments may be commented upon.

Table II. *Series* 1.

| Treatment—nitrate of soda at rate of 1 cwt. per statute acre, applied | Yield of grain per plot g. | Average number of tillers per plant at harvest | Grain total N (on dry matter) % | 1000-grain weight g. | Wt. of grain per Ear g. | Wt. of grain per Plant g. | Total number of plants per six plots, 1936, and seven plots, 1937 | Total number of ears per six plots, 1936, and seven plots, 1937 |
|---|---|---|---|---|---|---|---|---|
| | | | Spratt-Archer, 1936 | | | | | |
| *A* Control | 250·2 | 2·886 | 1·496 | 45·27 | 0·659 | 2·642 | 568 | 2274 |
| *B* At sowing | 358·2 | 3·972 | 1·452 | 45·82 | 0·720 | 3·570 | 601 | 2989 |
| *C* At appearance of first tiller | 332·3 | 3·774 | 1·440 | 46·04 | 0·715 | 3·422 | 583 | 2782 |
| *D* At erection of tillers | 299·5 | 3·908 | 1·481 | 44·75 | 0·614 | 3·014 | 596 | 2927 |
| *E* At flowering | 272·7 | 3·228 | 1·833 | 48·93 | 0·660 | 2·765 | 592 | 2497 |
| *F* 3 weeks after flowering | 269·7 | 2·855 | 1·693 | 46·94 | 0·731 | 2·813 | 576 | 2222 |
| Average | **297·08** | **3·437** | **1·566** | **46·29** | **0·683** | **3·038** | | |
| Sig. difference at 5% | **30·1** | **0·371** | **0·0425** | **1·007** | **0·0628** | **0·298** | | |
| Sig. difference at 1% | **41·1** | **0·506** | **0·0580** | **1·373** | **0·0857** | **0·406** | | |
| | | | Spratt-Archer, 1937 | | | | | |
| *A* Control | 137·4 | 0·946 | 1·661 | 39·23 | 0·714 | 1·408 | 687 | 1333 |
| *B* At sowing | 206·9 | 1·495 | 1·605 | 39·84 | 0·857 | 2·143 | 680 | 1700 |
| *C* At appearance of first tiller | 218·4 | 1·604 | 1·622 | 41·07 | 0·832 | 2·170 | 711 | 1848 |
| *D* At erection of tillers | 211·3 | 1·734 | 1·601 | 40·64 | 0·758 | 2·071 | 713 | 1943 |
| *E* At flowering | 145·9 | 0·912 | 2·039 | 41·56 | 0·775 | 1·493 | 683 | 1307 |
| *F* 3 weeks after flowering | 134·0 | 0·811 | 2·011 | 40·23 | 0·748 | 1·365 | 690 | 1250 |
| *G* At sowing and 3 weeks after flowering | 213·6 | 1·505 | 1·914 | 41·79 | 0·870 | 2·162 | 691 | 1727 |
| Average | **181·1** | **1·285** | **1·799** | **40·62** | **0·794** | **1·830** | | |
| Sig. difference at 5% | **18·0** | **0·225** | **0·0521** | **1·10** | **0·0955** | **0·243** | | |
| Sig. difference at 1% | **24·3** | **0·303** | **0·0702** | **1·48** | **0·129** | **0·327** | | |
| | | | Spratt, 1937 | | | | | |
| *A* Control | 149·1 | 0·851 | 1·749 | 41·54 | 0·814 | 1·509 | 693 | 1281 |
| *B* At sowing | 217·4 | 1·550 | 1·618 | 41·31 | 0·865 | 2·203 | 692 | 1764 |
| *C* At appearance of first tiller | 264·3 | 1·722 | 1·665 | 43·24 | 0·959 | 2·612 | 710 | 1928 |
| *D* At erection of tillers | 203·6 | 1·471 | 1·748 | 42·97 | 0·827 | 2·043 | 697 | 1721 |
| *E* At flowering | 156·6 | 0·884 | 2·055 | 42·27 | 0·856 | 1·621 | 680 | 1279 |
| *F* 3 weeks after flowering | 141·4 | 0·804 | 2·062 | 41·54 | 0·809 | 1·462 | 678 | 1222 |
| *G* At sowing and 3 weeks after flowering | 241·1 | 1·467 | 1·965 | 42·89 | 0·962 | 2·370 | 712 | 1759 |
| Average | **196·2** | **1·250** | **1·837** | **42·25** | **0·870** | **1·974** | | |
| Sig. difference at 5% | **15·5** | **0·127** | **0·0679** | **0·789** | **0·0584** | **0·159** | | |
| Sig. difference at 1% | **20·9** | **0·171** | **0·0914** | **1·06** | **0·0789** | **0·214** | | |

SERIES 1

The data under review in this series include the number of surviving plants and ear-bearing tillers, the yield of grain, the total nitrogen and 1000-grain weight per plot. The number of surviving tillers per plant was obtained by an actual count made at harvest as each plant was pulled up.

The results for the two years are given in Table II, the figures in each case being an average of six plots in the case of 1936, and of seven plots in 1937. For purposes of easier comparison by seasons the results are summarized in Table III, and the statistical significance of individual figures is shown graphically in Table IV, Figs. 1–5.

Table III. *Showing various results by treatments and years**

| Treatment and year | | Yield of grain per plot g. | Av. no. of tillers per plant at harvest | Total N % | 1000-grain weight g. | Weight of grain per ear g. | Weight of grain per plant g. |
|---|---|---|---|---|---|---|---|
| *A* | 1936 | 250·2 | 2·886 | 1·496 | 45·27 | 0·659 | 2·642 |
| | 1937 | 137·4 | 0·946 | 1·661 | 39·23 | 0·714 | 1·408 |
| | **1937** | **149·1** | **0·851** | **1·749** | **41·54** | **0·814** | **1·509** |
| *B* | 1936 | 358·2 | 3·972 | 1·452 | 45·82 | 0·720 | 3·570 |
| | 1937 | 206·9 | 1·495 | 1·605 | 39·84 | 0·857 | 2·143 |
| | **1937** | **217·4** | **1·550** | **1·618** | **41·81** | **0·865** | **2·203** |
| *C* | 1936 | 332·3 | 3·774 | 1·440 | 46·04 | 0·715 | 3·422 |
| | 1937 | 218·4 | 1·604 | 1·622 | 41·07 | 0·832 | 2·170 |
| | **1937** | **264·3** | **1·722** | **1·665** | **43·24** | **0·959** | **2·612** |
| *D* | 1936 | 299·5 | 3·908 | 1·481 | 44·75 | 0·614 | 3·014 |
| | 1937 | 211·3 | 1·724 | 1·601 | 40·64 | 0·758 | 2·071 |
| | **1937** | **203·6** | **1·471** | **1·748** | **42·97** | **0·827** | **2·043** |
| *E* | 1936 | 272·7 | 3·228 | 1·833 | 48·93 | 0·660 | 2·765 |
| | 1937 | 145·9 | 0·912 | 2·039 | 41·56 | 0·775 | 1·493 |
| | **1937** | **156·6** | **0·884** | **2·055** | **42·27** | **0·856** | **1·621** |
| *F* | 1936 | 269·7 | 2·855 | 1·693 | 46·94 | 0·731 | 2·813 |
| | 1937 | 134·6 | 0·811 | 2·011 | 40·23 | 0·748 | 1·365 |
| | **1937** | **141·4** | **0·804** | **2·062** | **41·54** | **0·809** | **1·462** |
| *G* | 1936 | — | — | — | — | — | — |
| | 1937 | 213·6 | 1·505 | 1·914 | 41·79 | 0·870 | 2·162 |
| | **1937** | **241·1** | **1·467** | **1·965** | **42·89** | **0·962** | **2·370** |
| | | | | *Average* | | | |
| | 1936 | 297·08 | 3·437 | 1·566 | 46·29 | 0·683 | 3·038 |
| | 1937 | 181·1 | 1·285 | 1·779 | 40·62 | 0·704 | 1·830 |
| | **1937** | **196·2** | **1·250** | **1·837** | **42·25** | **0·870** | **1·974** |
| | | | | *Significant Difference* | | | |
| 5% | 1936 | 30·1 | 0·371 | 0·0425 | 1·007 | 0·0628 | 0·298 |
| | 1937 | 18·0 | 0·225 | 0·0521 | 1·10 | 0·0955 | 0·243 |
| | **1937** | **15·5** | **0·127** | **0·0679** | **0·789** | **0·0584** | **0·159** |
| 1% | 1936 | 41·1 | 0·506 | 0·0580 | 1·373 | 0·0857 | 0·406 |
| | 1937 | 24·3 | 0·303 | 0·0702 | 1·48 | 0·129 | 0·327 |
| | **1937** | **20·9** | **0·171** | **0·0914** | **1·06** | **0·0789** | **0·214** |

* Figures for Spratt shown in heavier type.

Table IV. *Comparison of significance* ($\pm\pm$ *sig. at* 1 %; $\pm$ *sig. at* 5 %; 0 = *no significant difference*) *in favour of column heading.* (***Results for Spratt shewn in heavier type***)

| Treatment and year | | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|
| *A* | 1936 | 250·2 | + + | + + | + + | 0 | 0 | |
| | 1937 | 137·4 | + + | + + | + + | 0 | 0 | + + |
| | **1937** | **149·1** | **+ +** | **+ +** | **+ +** | **0** | **0** | **+ +** |
| *B* | 1936 | − − | 358·2 | 0 | − − | − − | − − | |
| | 1937 | − − | 206·9 | 0 | 0 | − − | − − | 0 |
| | **1937** | **− −** | **217·4** | **+ +** | **0** | **− −** | **− −** | **+ +** |
| *C* | 1936 | − − | 0 | 332·3 | − | − − | − − | |
| | 1937 | − − | 0 | 218·4 | 0 | − − | − − | 0 |
| | **1937** | **− −** | **− −** | **264·3** | **− −** | **− −** | **− −** | **− −** |
| *D* | 1936 | − − | + + | + | 299·5 | 0 | 0 | |
| | 1937 | − − | 0 | 0 | 211·3 | − − | − − | 0 |
| | **1937** | **− −** | **0** | **+ +** | **203·6** | **− −** | **− −** | **+ +** |
| *E* | 1936 | 0 | + + | + + | 0 | 272·7 | 0 | |
| | 1937 | 0 | + + | + + | + + | 145·9 | 0 | + + |
| | **1937** | **0** | **+ +** | **+ +** | **+ +** | **156·6** | **0** | **+ +** |
| *F* | 1936 | 0 | + + | + + | 0 | 0 | 269·7 | |
| | 1937 | 0 | + + | + + | + + | 0 | 134·6 | + + |
| | **1937** | **0** | **+ +** | **+ +** | **+ +** | **0** | **141·4** | **+ +** |
| *G* | 1936 | | | | | | | |
| | 1937 | − − | 0 | 0 | 0 | − − | − − | 213·6 |
| | **1937** | **− −** | **− −** | **+ +** | **− −** | **− −** | **− −** | **241·1** |

Fig. 1. Yield of grain.

| Treatment and year | | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|
| *A* | 1936 | 2·886 | + + | + + | + + | 0 | 0 | |
| | 1937 | 0·946 | + + | + + | + + | 0 | 0 | + + |
| | **1937** | **0·851** | **+ +** | **+ +** | **+ +** | **0** | **0** | **+ +** |
| *B* | 1936 | − − | 3·972 | 0 | 0 | − − | − − | |
| | 1937 | − − | 1·495 | 0 | +* | − − | − − | 0 |
| | **1937** | **− −** | **1·550** | **+ +** | **0** | **− −** | **− −** | **0** |
| *C* | 1936 | − − | 0 | 3·774 | 0 | − − | − − | |
| | 1937 | − − | 0 | 1·604 | 0 | − − | − − | 0 |
| | **1937** | **− −** | **− −** | **1·722** | **− −** | **− −** | **− −** | **− −** |
| *D* | 1936 | − − | 0 | 0 | 3·908 | − − | − − | |
| | 1937 | − − | −* | 0 | 1·724 | − − | − − | 0 |
| | **1937** | **− −** | **0** | **+ +** | **1·471** | **− −** | **− −** | **0** |
| *E* | 1936 | 0 | + + | + + | + + | 3·228 | − | |
| | 1937 | 0 | + + | + + | + + | 0·912 | 0 | + + |
| | **1937** | **0** | **+ +** | **+ +** | **+ +** | **0·884** | **0** | **+ +** |
| *F* | 1936 | 0 | + + | + + | + + | + | 2·855 | |
| | 1937 | 0 | + + | + + | + + | 0 | 0·811 | + + |
| | **1937** | **0** | **+ +** | **+ +** | **+ +** | **0** | **0·804** | **+ +** |
| *G* | 1936 | | | | | | | |
| | 1937 | − − | 0 | 0 | 0 | − − | − − | 1·505 |
| | **1937** | **− −** | **0** | **+ +** | **0** | **− −** | **− −** | **1·467** |

Fig. 2. Number of tillers per plant at harvest.

* Barely significant.

Table IV (*continued*)

| Treatment and year | | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|
| *A* | 1936 | 1·496 | – | – | 0 | + + | + + | |
| | 1937 | 1·661 | – | 0 | – | + + | + + | + + |
| | **1937** | **1·749** | **– –** | **–** | **0** | **+ +** | **+ +** | **+ +** |
| *B* | 1936 | + | 1·452 | 0 | 0 | + + | + + | |
| | 1937 | + | 1·605 | 0 | 0 | + + | + + | + + |
| | **1937** | **+ +** | **1·618** | **0** | **+ +** | **+ +** | **+ +** | **+ +** |
| *C* | 1936 | + | 0 | 1·440 | 0 | + + | + + | |
| | 1937 | 0 | 0 | 1·622 | 0 | + + | + + | + + |
| | **1937** | **+** | **0** | **1·665** | **+** | **+ +** | **+ +** | **+ +** |
| *D* | 1936 | 0 | 0 | 0 | 1·481 | + + | + + | |
| | 1937 | + | 0 | 0 | 1·601 | + + | + + | + + |
| | **1937** | **0** | **– –** | **–** | **1·748** | **+ +** | **+ +** | **+ +** |
| *E* | 1936 | – – | – – | – – | – – | 1·833 | – – | |
| | 1937 | – – | – – | – – | – – | 2·039 | 0 | – – |
| | **1937** | **– –** | **– –** | **– –** | **– –** | **2·055** | **0** | **–** |
| *F* | 1936 | – – | – – | – – | – – | + + | 1·693 | |
| | 1937 | – – | – – | – – | – – | 0 | 2·011 | – – |
| | **1937** | **– –** | **– –** | **– –** | **– –** | **0** | **2·062** | **– –** |
| *G* | 1936 | | | | | | | |
| | 1937 | – – | – – | – – | – – | + + | + + | 1·914 |
| | **1937** | **– –** | **– –** | **– –** | **– –** | **+** | **+ +** | **1·965** |

Fig. 3. Grain, total nitrogen %.

| Treatment and year | | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|
| *A* | 1936 | 45·27 | 0 | 0 | 0 | + + | + + | |
| | 1937 | 39·23 | 0 | + + | + | + + | 0 | + + |
| | **1937** | **41·54** | **0** | **+ +** | **+ +** | **0** | **0** | **+ +** |
| *B* | 1936 | 0 | 45·82 | 0 | – | + + | + | |
| | 1937 | 0 | 39·84 | + | 0 | + + | 0 | + + |
| | **1937** | **0** | **41·31** | **+ +** | **+ +** | **+** | **0** | **+ +** |
| *C* | 1936 | 0 | 0 | 46·04 | – | + + | 0 | |
| | 1937 | – – | – | 41·07 | 0 | 0 | 0 | 0 |
| | **1937** | **– –** | **– –** | **43·24** | **0** | **–** | **– –** | **0** |
| *D* | 1936 | 0 | + | + | 44·75 | + + | + + | |
| | 1937 | – | 0 | 0 | 40·64 | 0 | 0 | + |
| | **1937** | **– –** | **– –** | **0** | **42·97** | **0** | **– –** | **0** |
| *E* | 1936 | – – | – – | – – | – – | 48·93 | – – | |
| | 1937 | – – | – – | 0 | 0 | 41·56 | – | 0 |
| | **1937** | **0** | **–** | **+** | **0** | **42·27** | **0** | **0** |
| *F* | 1936 | – – | – | 0 | – – | + + | 46·94 | |
| | 1937 | 0 | 0 | 0 | 0 | + | 40·23 | + + |
| | **1937** | **0** | **0** | **+ +** | **+ +** | **0** | **41·54** | **+ +** |
| *G* | 1936 | | | | | | | |
| | 1937 | – – | – – | 0 | – | 0 | – – | 41·79 |
| | **1937** | **– –** | **– –** | **0** | **0** | **0** | **– –** | **42·89** |

Fig. 4. 1000-grain weight.

Table IV (*continued*)

| Treatment and year | | *A* | *B* | *C* | *D* | *E* | *F* | *G* |
|---|---|---|---|---|---|---|---|---|
| *A* | 1936 | 0·659 | 0 | 0 | 0 | 0 | + | |
| | 1937 | 0·714 | + + | + | 0 | 0 | 0 | + + |
| | **1937** | **0·814** | **0** | **+ +** | **0** | **0** | **0** | **+ +** |
| *B* | 1936 | 0 | 0·720 | 0 | – – | 0 | 0 | |
| | 1937 | – – | 0·857 | 0 | – | 0 | – | 0 |
| | **1937** | **0** | **0·865** | **+ +** | **0** | **0** | **0** | **+ +** |
| *C* | 1936 | 0 | 0 | 0·715 | – – | 0 | 0 | |
| | 1937 | – | 0 | 0·832 | 0 | 0 | 0 | 0 |
| | **1937** | **– –** | **– –** | **0·959** | **– –** | **– –** | **– –** | **0** |
| *D* | 1936 | 0 | + + | + + | 0·614 | 0 | + + | |
| | 1937 | 0 | + | 0 | 0·758 | 0 | 0 | + |
| | **1937** | **0** | **0** | **+ +** | **0·827** | **0** | **0** | **+ +** |
| *E* | 1936 | 0 | 0 | 0 | 0 | 0·660 | + | |
| | 1937 | 0 | 0 | 0 | 0 | 0·775 | 0 | 0 |
| | **1937** | **0** | **0** | **+ +** | **0** | **0·856** | **0** | **+ +** |
| *F* | 1936 | – | 0 | 0 | – – | – – | 0·731 | |
| | 1937 | 0 | + | 0 | 0 | 0 | 0·748 | + |
| | **1937** | **0** | **0** | **+ +** | **0** | **0** | **0·809** | **+ +** |
| *G* | 1936 | | | | | | | |
| | 1937 | – – | 0 | 0 | – | 0 | – | 0·870 |
| | **1937** | **– –** | **– –** | **0** | **– –** | **– –** | **– –** | **0·962** |

Fig. 5. Weight of grain per ear.

Before proceeding to a detailed examination of the figures the general weather conditions and other features likely to affect the results may be noted.

The early months of 1936 were cold and wet, and the soil conditions at seeding time were only moderately favourable. The weather during May and June was no more than fairly favourable, a long period with low temperatures in the former month greatly restricting growth. July was persistently wet, and these conditions continued until harvest in about the middle of August.

The autumn of 1936 and the early months of 1937 were characterized by a phenomenally heavy rainfall. Sowing in 1937 was delayed until after the middle of April, and the physical condition of the soil at that time was thoroughly unsatisfactory. Growth in the early stages was slow, and imposed on this condition there was a period of unfavourable cold, wet weather in June. July on the whole was more favourable, and following this the dry sunny weather of August effected a most beneficial change in the "quality" of barley.

## Discussion of results

### (1) *Yield of grain*

An outstanding feature of the grain yield was the much lower level of 1937 compared with that of 1936, the control of Spratt-Archer in 1937, for example, being only 54·8% of the corresponding plots in 1936. But notwithstanding this seasonal difference it will be observed that in both years and with both varieties the treatments *B*, *C* and *D* were significantly superior to the control at the 1% point, and that neither variety in either year exhibited a superiority to the control at that point in treatments *E* and *F*.

Treatment *G* was superior to the control at the 1% point in the case of both varieties.

The differences between the treatments *B*, *C*, and *D* were less regular; it will be observed, however, that in the case of Spratt treatment *C* was significantly superior to *B* and *D* at the 1% point, whilst *B* in the case of Spratt-Archer was equal to *C* in both years, superior to *D* in 1936 and equal to that treatment in 1937. Treatment *G*, it will be observed, was equal to *B*, *C* and *D* but significantly superior to *E* and *F* in the case of Spratt-Archer; with Spratt, on the other hand, *G* is significantly inferior to *C* but superior to all other treatments.

Thus, while treatment *B* may be regarded as exhibiting an optimum value in Spratt-Archer in 1936 it did not exhibit a similar superiority to *C* and *D* in 1937; with Spratt, on the other hand, treatment *C* was the one of optimum value.

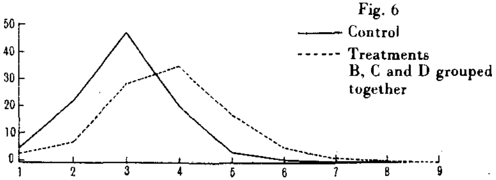
### (2) *Number of tillers per plant at harvest*

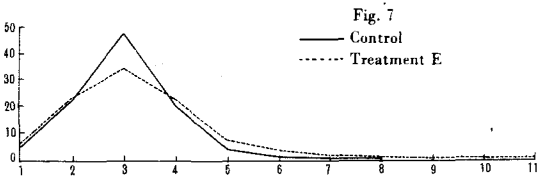
The close relation of surviving tillers at harvest to the yield of grain is demonstrated in Figs. 17–19, p. 491. Again, the results assume the aspect of a comparison of the three treatments *B*, *C*, *D* and the two treatments *E*, *F*—in other words, a comparison of those treatments which exhibited a significant difference to the control at the 1% point and those which equalled the control. These differences, it will be observed, are established between sets of plots which received nitrogen at different phases before flowering and those which received it at or subsequent to flowering.

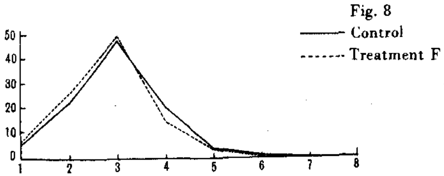
The general trend resulting from the various treatments is illustrated by Figs. 6–16 in which the number of plants with, respectively, one, two, etc., tillers per plant, is shown as a percentage of the total number of plants per treatment.

Between the three treatments *B*, *C* and *D* themselves, the differences were very regular in Spratt-Archer, but in Spratt treatment *C* was definitely greater than the other two.

Treatment *G* deserves special comment; as was expected, this treatment was at least equal to *B* in both varieties, but in Spratt treatment *C* was superior to *G*, thereby furnishing a legitimate reason for the significant difference found in the total grain yield. The significant differences existing between *G*, *E* and *F* follow as a natural sequence.

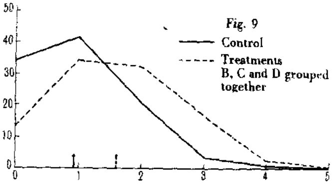


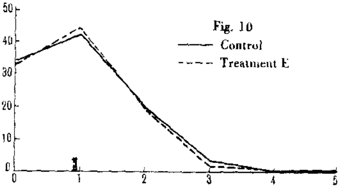



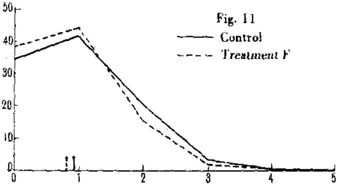



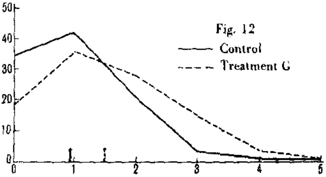

Spratt-Archer, 1936.

**Figs. 6–8. Showing the number of plants with respectively 1, 2, 3 etc. ear-bearing tillers at harvest as a percentage of the total number of plants per 6 plots of the control, and of the treatments.**



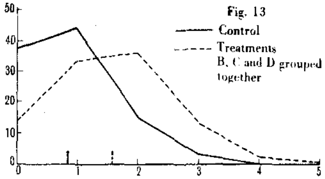



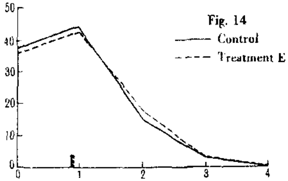



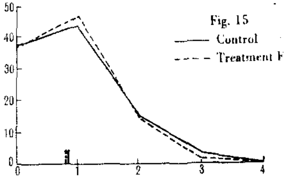



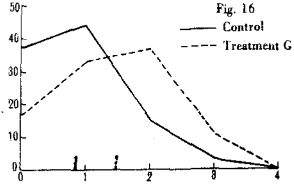

Spratt-Archer, 1937

Figs. 9–12. Showing the number of plants with respectively 1, 2, 3 etc. ear-bearing tillers at harvest as a percentage of the total number of plants per 7 plots of the control, and of the treatments. The arrows indicate the mean in each case.




Spratt, 1937

Figs. 13–16. Showing the number of plants with respectively 1, 2, 3 etc. ear-bearing tillers at harvest as a percentage of the total number of plants per 7 plots of the control and of the treatments. The arrows indicate the mean in each case.

(3) *Total nitrogen content of the grain*

The results under this heading again fall into two groups, namely, those significantly superior to the control, and those exhibiting either equality with the control or a small difference therefrom. It will be noted that a division on this basis is again one following the lines of pre-flowering and post-flowering treatment. Thus, *E*, *F*, throughout, exhibited a constant significant difference to the control at the 1% point whilst *B*, *C*, *D* were irregular in their behaviour. The only differences in the latter cases which were significant at the 1% point were *B* treatment in Spratt which was less than the control, and *D* treatment in the same variety which was superior to *B* treatment.

It will be observed that the percentage of nitrogen in *G* treatment in both varieties was higher than the control, but in Spratt-Archer it was less than both *E* and *F* at the 1% point; in Spratt, however, while less than *F* at the 1% point, it was only inferior to *E* at the 5% point. This result thus provides additional confirmation of the original premise that the number of ears surviving at harvest may be an index of quality, as it is of yield, in barley.

(4) 1000-*grain weight*

The differences under this heading were much less regular than in those preceding. The outstanding feature in 1936 was the superiority of *E*, *F* to the control, and the equality of *B*, *C*, *D* therewith. Further, *E* in that year was superior to *F*. In 1937 and with the same variety, *C*, *E*, *G* were superior to the control at the 1%, and *D* at the 5% point. With Spratt significance was obtained with *C*, *D* and *G* but not with either *E* or *F*, and both *C* and *D* were significantly superior to *F* at the 1% point.

This feature will be further dealt with when considering the results under series 2.

(5) *Weight of grain per ear*

It will be observed that whilst the 1000-grain weight in 1936 was superior in all treatments to that of 1937, the weight of grain per ear assumed an exactly contrary condition. This seems to imply that although the 1000-grain weight in 1936 was higher than in 1937 it was not sufficient to counter-balance the decreased weight of grain consequent on a very much increased number of surviving ears per plant. In 1936 it was only in *F* that there was a small difference in excess of the control and in this case the total grain yield was only equal to the control. The equality

of *B*, *C*, *D* with the control in 1936 was further evidence of the reliance of varieties in this country on number of surviving ears rather than on size of ears for superiority in total grain yield, for *B*, *C*, *D* were significantly superior to the control in this respect.

## SERIES 2

Table V below shows the average number of surviving plants, the yield of grain, nitrogen percentage, 1000-grain weight, and weight of grain per plant for each of the five treatments and the control in this series.

Table V

| Treatment | Yield of grain per plot g. | Grain, total N % | 1000-grain weight g. | Weight of grain per plant g. | Number of surviving plants per plot |
|---|---|---|---|---|---|
| *a* | 269·8 | 1·560 | 45·87 | 2·764 | 98 |
| *b* | 96·3 | 2·005 | 55·85 | 1·220 | 77 |
| *c* | 115·2 | 1·962 | 55·28 | 1·264 | 91 |
| *d* | 117·3 | 1·946 | 54·20 | 1·247 | 94 |
| *e* | 101·8 | 1·956 | 49·97 | 1·097 | 92 |
| *f* | 104·3 | 1·969 | 50·39 | 1·121 | 93 |

In this series the number of plants at harvest was uniform except in treatment *b*, in which case the reduced number of snrviving plants may be attributed to the unavoidable manipulation consequent on the continual abscission of tillers. The average yield of grain per plot of the five treated lots in comparison with the control was not unexpected, but the total nitrogen content and the 1000-grain weight were not so clearly foreseen. It will be observed that in comparison with the control all treatments exhibited an increase in 1000-grain weight which varied from a minimum increase of approximately 9% in treatment *e*, to a maximum of 21·5% in treatment *b*. The difference between the control and all other treatments in 1000-grain weight is significant at 1%, but whilst *B*, *C*, and *D*, respectively, differ significantly from *E* and *F*, the differences between the former three and between the latter two treatments are not significant.

The total nitrogen content is similar for the five treatments, and on the average shows an increase of 28% over the control. In every case the treatments are significantly greater than the control, but there is no significant difference amongst treatments themselves.

The two sets of treatments *e* and *f* demand special attention, since both exemplify a condition analogous to the control up to the date of flowering, and consequently to a stage considerably in advance of *b*, *c* and *d*, respectively. Nevertheless, both *e* and *f* exhibited a higher total

nitrogen content and approximately 10% higher 1000-grain weight than the control, both of which differences arose from causes operating subsequent to flowering.

It will also be observed in regard to *e* and *f* that the final abscission in each case was effected on the same day but that although both the straw and the ears of tillers were removed in the case of *e*, and only the ears in *f*, there was nevertheless no difference in the total nitrogen and 1000-grain weight of these two treatments.

These results do not dispose of the general question of the translocation of material between tillers of the same plant, but they indicate the character of the differences likely to be found under identical environmental conditions when tillering, owing to various causes, is reduced. In series 1 it has been shown that a supply of nitrogen to the plant at or subsequent to flowering may increase both the nitrogen content and 1000-grain weight, and since both of these features were evident in treatment *e*, it might be assumed that in that particular case they arose as a result of the operation of a like cause.

Smith (1933) has shown that the possibility of exchange between tillers exists under some circumstances, but to what extent exchange is affected under normal conditions is still undetermined. In the present case had it not been for the result obtained in treatment *e* there would have been some justification for regarding the increased nitrogen content and 1000-grain weight in treatment *f* as effects arising from the translocation of material from the fully developed but earless tillers to the main shoot. Since, however, both the total nitrogen and 1000-grain weight are closely similar in *e* and *f* treatments this deduction is invalid, although the source of the additional nitrogen found in treatment *e* remains a matter for further investigation. The position in relation to treatment *e* does, however, recall the extraordinary return of vegetative development exhibited by barley stubbles in 1921. In that very hot and rainless summer the growth after harvest was so abundant as to indicate very heavy shedding. There was, however, insufficient moisture at that time to promote germination, and on examination it was found that the young green shoots arose from the old plants which, since no moisture had fallen after harvest, must have provided the material for growth from their own substance. This indicates the possibility of the incomplete depletion of the material of the lower portions of the straw, and probably of the roots, which may, under conditions comparable with those operating in this series, then furnish material for translocation to the grain.

## DISCUSSION

The effect of the application of nitrogen in these investigations must be viewed in strict relation to the time of application both in regard to the yield and nitrogen content of the grain. The early applications, namely, *B*, *C* and *D*, in both years and with both varieties resulted in an increase in yield of grain and no concurrent increase in total nitrogen. These applications thus conform with results usually obtained in normal agricultural practice.

The applications made at or subsequent to flowering did not result in a significant enhancement of the grain yield, but the nitrogen content of the grain was very considerably increased. These results are in agreement with the findings of Davidson & Le Clerc (1917), who, by applying nitrate of soda to wheat at heading time, were able to produce an improved quality of grain with reference to colour and protein content, without in any way affecting vegetative growth. Gericke (1920) also showed that the protein content of wheat which received nitrogen 110 days after planting exhibited an increase of 77% over that which received a similar quantity of nitrogen at planting.

With the early applications of nitrogen there was a substantial and significant increase in the number of ears surviving at harvest, but no increase in the number of surviving ears when the applications were made at and subsequent to flowering.

At the stage in the life history of the cereal plant at which it passes from a period of active vegetative development to seed formation, indicated first by the tillers assuming a strictly vertical position and then followed rapidly by flowering, there is a decided cessation in effective tiller formation, as measured by the number of ears surviving at harvest. Subsequent to flowering the effect of nitrogen is more restricted, and from this stage onwards is exhibited in a higher total nitrogen content in the grain, and, under some conditions, by a higher 1000-grain weight. Even at the advanced stage exemplified in treatment *F* there is evidence of the absorption of nitrogen from the soil.

The increase in grain yield under treatments *B* and *C*, although obtained concurrently with a higher ear survival, was not found in conjunction with a reduction in weight of grain per ear, nor was there a regular trend in either an upward or downward direction in the 1000-grain weight. With treatment *D*, however, there was evidence of a fall in yield as compared with *B* which can be accounted for by a reduction in the weight of grain per ear, and this in turn by a slight

decrease in 1000-grain weight. Thus, in treatment $D$ although the number of surviving ears was equal to that found under $B$ it may be inferred that the application of nitrogen synchronized with a stage at which it was just possible to ensure the survival of some tillers which would otherwise have failed to contribute anything to the yield of grain.

Engledow & Wadham (1924) have shown that the ear and straw of successive tillers, denominated $T_0$, $T_1$, $T_2$ *et seq.*, exhibited a gradation in weight. It is thus highly probable that the tillers most influenced by the application of nitrogen at tiller erection stage are the latest formed, and that these, by reason of their position in the succession, would be the lowest in ear weight at the time the manure was applied.

Engledow & Ramiah (1930, p. 301) concluded from a study of the time incidence of the critical period in wheats that "It must, therefore, be recognized that very early tillering varieties, while producing a high average number of ears per plant at harvest, inevitably tend to produce relatively small ears on their side tillers." At another point in their paper, the same investigators state (p. 325): "Size of ear, both in number of grains and average grain weight, decreases in order of time of formation of the stem. Moreover, average ear size increases with number of ears per plant."

The significance of these observations, at first sight contradictory, is clearly exemplified throughout in treatments $B$, $C$, $D$. The general tendency towards relatively small ears in plants producing high average number of ears per plant is shown by treatment $D$, which in point of time is a near approximation to the critical period, and thus exemplifies a physiological condition much in advance of that represented by $B$ and $C$; on the other hand, if $B$ and $C$ are compared with the control the results furnish ample justification of the conclusion that the average ear size increases with the number of ears per plant.

In view of the relationship of high ear survival, high yield of grain and low total nitrogen content (vide Figs. 17–19), the results of treatment $G$ assume added importance especially in regard to total nitrogen, since in this case a position of close similarity to treatment $B$ was reached in the number of surviving ears per plant before the second application of nitrogen was made on the same date as to treatment $F$. Comparing $E$ and $F$, respectively, with $G$ the differences in nitrogen content were significant at the 1% point in three cases and at the 5% point in one case, and thus furnish additional corroboration of the general proposition that the number of surviving ears may form an index of decreasing nitrogen content. But the actual nitrogen content apart from the

Fig. 17

Spratt-Archer, 1936

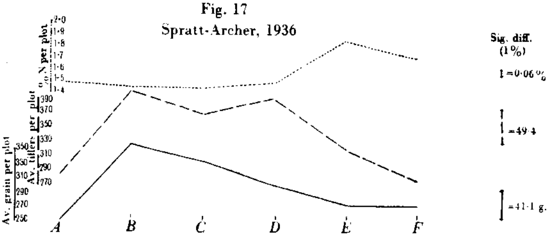



Fig. 18

Spratt-Archer, 1937

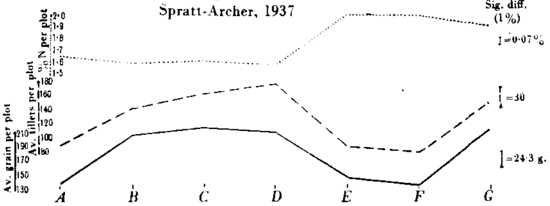
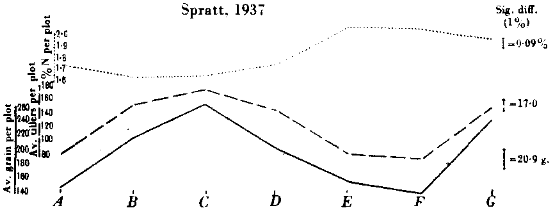



Fig. 19

Spratt, 1937




Figs. 17–19. Showing the average yield of grain, number of ear-bearing tillers and percentage total nitrogen per plot for the control and treatments, 1936 and 1937. Significant differences at 1% are indicated at the side of each figure.

varietal effect will depend on the extent to which nitrogen is absorbed from the soil during the period between flowering and full ripeness. In the light of this proviso the difference between the results obtained with an isolated plant and a high tillering plant grown under conditions of greater competition can be explained as differences arising from a supply of nitrogen available to the plant over a protracted period in the one case, and during a more limited period in the other. Both conditions may have been such as to encourage abundant vegetative development in the early stages, but whilst in one nitrogen was available concurrently with a declining rate of tillering, in the other a decrease in the supply of nitrogen synchronized with the gradual reduction of vegetative activity.

Thus, whilst the results under immediate review indicate that the number of surviving ears may be an index of low total nitrogen content in the grain, this criterion can only be usefully employed comparatively under conditions which postulate strict equality of nitrogen supply subsequent to flowering.

With regard to the 1000-grain weight in its relation to the total nitrogen of the grain: Johannsen (1899) in his researches on Goldthorpe barley showed that the larger grains of the ear had a lower percentage of nitrogen than the smaller grains of the same ear. These comparisons, however, were made with grain produced under the closest approach to one environment. They cannot be applied in an absolute sense, or to the results discussed in this paper, which are based on figures representing average conditions of a number of plants drawn from a series of separate plots.

Kiessling (1915) demonstrated the hereditary character of 1000-grain weight in two barleys he studied, and found that the average nitrogen content and average corn weight were positively correlated. In other experiments, however, he established positive, negative and insignificant correlations between the two attributes, although positive correlations were more common than negative ones.

The differences observed in the results under immediate review illustrate one of the conditions operating to produce high grain weight associated with high nitrogen content. In series 1 the condition was a supply of nitrogen subsequent to flowering, when the formation of tillers capable of producing ears at harvest had ceased. The influence of the abscission of tillers (series 2) on the 1000-grain weight and total nitrogen was exactly similar in direction, and may be interpreted as arising from a like cause. In the latter experiments (Table V), the various treatments produced less than a half of the grain obtained from the control, but

since presumably the same amount of nitrogen was available as in the control, the surplus over that required to produce one ear-bearing stem remained available, and was gradually absorbed throughout the full life history of the plant, and consequently subsequent to flowering. It has been noticed, however, that where the tillers were not removed until flowering commenced the effect was to reduce the 1000-grain weight and the weight of grain per ear in comparison with those of the earlier treatments; the total nitrogen content, nevertheless, was unaffected. Treatment *e*, series 2, however, is difficult to explain since the ears were not removed until flowering stage, by which time, it is presumed, most of the available soil nitrogen had been absorbed by the plant. It was anticipated, therefore, that whilst the final nitrogen figure might be in excess of the control, it would approximate more nearly to that figure than to those of the other treatments.

The association of 1000-grain weight with both high and low total nitrogen relative to the control in these investigations is in consonance with the results of other investigations (Hunter, 1926; Russell & Bishop, 1933), but its incidence in this case is directly attributable to the effect of nitrogen applied either previously to flowering, when it was found in conjunctiou with a relatively low nitrogen content, or at or subsequently to flowering when it was found in association with a relatively high nitrogen content.

To a considerable extent, therefore, these results conform with the conditions of both positive and negative correlation which Kiessling established in certain of his investigations.

Moreover, since the treatments which received nitrogenous fertilizer at various times before flowering produced significantly higher yields of grain and higher numbers of surviving ears than the control, it may be concluded that the 1000-grain weight was not adversely affected by the greater number of tillers produced per plant. It will be recollected at the same time that in series 2 the highest 1000-grain weights were obtained with the treatments made before flowering.

Thus it may be concluded that high 1000-grain weight when in association with a low total nitrogen is an indication of optimum conditions of growth for malting barley, i.e. conditions favouring high grain yield and low total nitrogen. High 1000-grain weights in association with high total nitrogen may also be found in conjunction with both high and low grain yield. In the former case it will probably exist with high tillering, and the high nitrogen is a result of the post-flowering absorption of nitrogen (see *G* plots, series 1), and in the latter the high

nitrogen occurs as a result of low tillering combined possibly with a condition of post-flowering absorption.

Finally, it is evident that whilst the 1000-grain weight may be an indication of *inter*-varietal differences, and in an *intra*-varietal sense of different cultural conditions, such as those described above, it is in itself no index of the total nitrogen content of the grain.

For reasons already stated a comparison of results on a varietal basis has not been attempted; nevertheless the very marked response of Spratt in treatment C should be noted, for it may indicate a strictly differential response, and thus a significant feature in agricultural practice. Again, but with the same limitation of application, it will be observed that in average yield, 1000-grain weight, and total nitrogen content Spratt is in advance of Spratt-Archer.

The results obtained up to the present do not dispose of the tillering question when viewed on a varietal basis; they merely indicate that in varieties of the Spratt-Archer type, when grown under a specified environment, high ear survival is an index of yield and quality, i.e. of low total nitrogen content. They also indicate that under conditions of normal agricultural practice high actual ear survival tends to indicate the lowest possible total nitrogen content in the grain for the particular environment in which any given crop is grown. But, when once high ear survival is secured as a definite varietal characteristic its utilization to the fullest extent will depend on the fertility of the soil on which a crop is grown.

From the breeding point of view the fundamental issue is the causation of high ear survival, more particularly of high ear survival relative to the number of tillers formed during the vegetative stage of growth. From studies of wheat varieties in New Zealand, Frankel (1935) has shown that this may be largely a matter of environment, for in New Zealand the predominant varieties are those with low initial tillering but high ear survival capacities, whereas in Great Britain the most productive varieties are those exhibiting both high initial tillering and high ear survival.

A further subject for investigation is that of relatively lower yielding varieties, such as those mentioned previously, which produce grain with low total nitrogen; it is hoped to include one of these in the trials to be carried out in 1938.

## SUMMARY

1. The high grain-yielding capacity and low total nitrogen content of predominant British varieties of barley, in conjunction with high ear survival at harvest, are discussed.

2. The results of a series of quantitative comparisons in which increased tillering was promoted by the application of nitrate of soda at various phases of plant development, and similarly when tillers were removed at various stages, were examined.

3. It was shown that the effect of nitrogen on the yield and quality of the grain depends upon the time in relation to the stage of plant development at which it was applied; the early applications enhanced the yield without detriment to the quality of the grain, whilst the later applications did not increase the yield but increased the total nitrogen content, and consequently reduced the malting quality of the grain.

4. The increase in yield was obtained primarily by an increase in the number of surviving tillers and to a less extent by an increase in yield of grain per ear; the latter was in some cases accompanied by an increase in 1000-grain weight. High ear survival in varieties such as those under examination may consequently be regarded as an index of high yield of grain and of low total nitrogen content.

5. The removal of tillers resulted in increased total nitrogen content and increased 1000-grain weight in the grain of the main stem; the former was not affected by the stage of development of the plant at which abscission was made, but the highest value of the latter was obtained when the abscission was made before flowering.

6. The relation of 1000-grain weight and nitrogen content was discussed, and it was shown that high 1000-grain weight may exist with both high and low total nitrogen. Although the 1000-grain weight is an hereditary attribute it is subject to considerable fluctuation and cannot by itself be usefully employed as an index of nitrogen content, and consequently of malting quality, in either an *inter-* or *intra*-varietal sense.


The writer gladly acknowledges the assistance of Dr H. O. Hartley in the statistical treatment of results, and of Messrs M. C. Buck, J. D. Palmer and K. P. Hedge in carrying out tiller counts, threshing and weighing samples, and in the various analytical determinations made in the course of the investigation.

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## STATISTICAL STUDY

(i) *Introduction*. Dr Hunter has kindly asked the author to supplement his paper by a statistical discussion of some of the results of his investigation. Details of the experiments are given in the text of the paper, so that it will suffice here to describe briefly their respective objects and designs.

In 1936 two experiments were carried out. In one of them, a manurial trial, nitrate of soda was applied at various stages in plant development, and the effect of various degrees of tillering resulting from these applications on characteristic plant attributes was examined. The second experiment was designed to study the effect of the abscission of tillers at different stages of their development on the quality and quantity of the grain of the main stem. Both experiments were designed as $6 \times 6$ Latin squares, the variety grown being Spratt-Archer.

The manurial trial was then repeated in 1937; but the experience gained during the 1936 trials suggested the incorporation of an additional treatment (a double application of manure) so that in 1937 two trials were designed as $7 \times 7$ Latin squares. In one of them the same variety, Spratt-Archer, was grown, whilst the other trial was laid out with Spratt.

The analysis of a Latin square design is now commonplace, so that details need not be given here. There are, however, a few points of interest concerning the manurial trials which made a special statistical investigation necessary, and these will be described briefly in this note.

(ii) *Significance and consistency of treatment differences.* The outstanding statistical feature of these trials is that the treatments had a definite and strong effect. In each case treatment differences were returned as highly significant by the $z$-test, so that the treatment averages (set out in Table III, p. 478) could be compared by the ordinary $t$-test. The results of these tests are set out in Table IV, pp. 479–81.

It will be seen from these tables that the significance of some of the differences is shown with almost perfect consistency in all three experiments, whilst other differences reveal inconsistencies. Thus, it will rightly be asked whether the significance of some of the differences might not depend on

(*a*) the weather and general conditions in a certain year, and

(*b*) the varieties used for these experiments.

The answer to question (*a*) can, of course, only be given by repetitions of the same experiment, and a test on this point is given by comparing treatment differences with the interaction between treatments and seasons. Such a test may be carried out for the variety Spratt-Archer. Discarding treatment $G$, which has been applied in 1937 only, we may regard the remaining treatment averages from the two Latin squares as two "Blocks" (seasons 1936 and 1937) containing six "plots" each (viz. the respective treatment averages). The analysis for two of the plant attributes (percentage nitrogen and average grain per plant) is set out in Tables IA and IIA:[1] only those differences which are shown with consistency in Table IV, p. 479, are confirmed as significant by this test.

Table IA. *Percentage nitrogen. Analysis of variance*

| Source of variation | D.F. | Mean square | |
|---|---|---|---|
| Treatments | 5 | 0·067107 | Sig. at 1% |
| Seasons | 1 | 0·109157 | Sig. at 1% |
| Error | 5 | 0·002341 | |

Table IIA. *Average grain per plant. Analysis of variance*

| Source of variation | D.F. | Mean square | |
|---|---|---|---|
| Treatments | 5 | 0·278424 | Sig. at 1% |
| Seasons | 1 | 4·782981 | Sig. at 1% |
| Error | 5 | 0·016457 | |

*Treatment averages*

| | | | | | | | Sig. diff. at | |
|---|---|---|---|---|---|---|---|---|
| Treatment ... ... | $A$ | $B$ | $C$ | $D$ | $E$ | $F$ | 5% | 1% |
| Percentage nitrogen | 1·578 | 1·528 | 1·531 | 1·541 | 1·936 | 1·852 | 0·124 | 0·195 |
| Average grain per plant | 2·025 | 2·856 | 2·796 | 2·542 | 2·129 | 2·089 | 0·330 | 0·517 |

[1] "A" added to Table numbers refers to Tables of the Statistical Study whilst ordinary Table numbers refer to Tables of the paper.

Although a single repetition of an experiment can hardly be regarded as sufficient to yield a satisfactory decision on the effect of seasonal conditions (also, there are only 5 degrees of freedom for error) it is of interest to note that conditions during the two years were very different. This difference (returned as highly significant in the above tests) is reflected in the large discrepancy between the respective yields, and the respective average numbers of surviving tillers per plant. Thus, treatment differences which are returned as significant by the above test have stood the test under markedly different general conditions.

As regards question (*b*) the information available is of course insufficient in so far as up to the present time only two varieties have been under consideration and also because the design of the experiment does not provide for a statistical comparison of the varieties.

The author has purposely set out to study his main problem for single varieties of special interest.

(iii) *Experimental error and nature of the distributions.* The fact that the soil conditions represented by the different treatments are most essential in determining the percentage nitrogen of the grain is also reflected in the remarkably low percentage error of this plant attribute in all three experiments. These percentage errors are set out together with those of other plant attributes in Table III A. It will be seen that in

Table III A. *Giving the experimental errors as percentages of the respective means for different plant attributes as shown below. (Manurial trials)*

| Attribute ... | | Av. tillers per plant | % nitrogen | Av. grain per plant | Total grain | Grain per ear | 1000-corn weight |
|---|---|---|---|---|---|---|---|
| Season | Variety | | | | | | |
| 1936 | Spratt-Archer | 8·97 | 2·25 | 8·10 | 8·42 | 7·62 | 1·81 |
| 1937 | Spratt-Archer | 16·04 | 2·69 | 12·14 | 9·12 | 11·02 | 2·47 |
| 1937 | Spratt | 9·32 | 3·39 | 7·38 | 7·24 | 6·15 | 1·71 |

addition to the nitrogen percentage there is a second plant attribute with a low percentage error: the 1000-corn weight. This, however, is not surprising; for a single one of these weight records may be regarded as the mean of the weights of 1000 single corns if the weights of the latter are expressed in milligrams. With Spratt-Archer the percentage error of the average number of surviving tillers per plant is much lower in 1936 than in 1937. This difference has been caused by the low average number of tillers in 1937 which is less than half that in 1936. The corresponding standard errors are 0·308 and 0·206 for 1936 and 1937 respectively so that the smaller standard error in 1937 concurs with the smaller average number of tillers per plant in that year. This would be expected for this plant attribute, for its average in 1937 is very near to 0,

the natural lower limit of its range. Indeed, the frequency polygons given in the paper (pp. 483–5) seem to indicate that this plant attribute tends to be distributed according to the Poisson law rather than the normal law of error. This would explain a correlation between mean and standard error. Some care is, therefore, necessary when applying the "Analysis of Variance technique" to such data. Recent sampling investigations (Eden & Yates, 1933; Hey, 1938), however, have shown that such deviations from normality do not affect seriously standard tests such as the $t$- and $z$-tests.[1] We are therefore justified in using the analysis of Variance technique in investigations connected with this plant attribute.

(iv) *Correlations.* There are two types of correlation coefficients which have to be considered in these experiments:

(A) The correlation between two plant attributes (percentage of nitrogen in the grain and 1000-corn weight say) determined for plots treated alike.

(B) The correlation between the average values of two plant attributes obtained on different treatments (treatment averages).

Table IV A. *Correlation coefficients between pairs of plant attributes calculated from the experimental data as shown below*

| Type of correlation | Variety | Year | Plant attributes: % nitrogen × av. number of tillers | % nitrogen × av. grain per plant | Av. number of tillers × av. grain per plant | Degrees of freedom |
|---|---|---|---|---|---|---|
| *A* | Spratt-Archer | 1936 | −0·077 | −0·334 | 0·506* | 19 |
| *A* | Spratt-Archer | 1937 | 0·014 | −0·360 | 0·367* | 29 |
| *A* | Spratt | 1937 | −0·229 | −0·294 | 0·643† | 29 |
| *B* | Spratt-Archer | 1936 | −0·572 | −0·587 | 0·838* | 4 |
| *B* | Spratt-Archer | 1937 | −0·585 | −0·544 | 0·958† | 5 |
| *B* | Spratt | 1937 | −0·653 | −0·529 | 0·968† | 5 |
| *B* | All three experiments | | −0·620† | −0·544* | 0·909† | 16‡ |

* Significant at 5%. † Significant at 1%.
‡ These contain two degrees of freedom for differences between the three regressions of the single experiments.

In Table IV A are given the correlation coefficients (of type (A)) as well as of type (B) for each of the three combinations of three plant attributes, viz. the percentage nitrogen, the average number of surviving tillers per plant and the average grain per plant. It is obvious from this table that the correlations of type (A) (calculated from the respective residual lines) are much smaller than the corresponding

[1] In fact one of the populations, which has been dealt with in one of the above papers, consisted of the number of ears in each of 7200 6 in. single row lengths of wheat.

correlation coefficients of type (B). Most of the former are insignificant although they are based on a reasonable number of degrees of freedom. It is true, some of the correlations of type (B) are insignificant too (because of the small number of degrees of freedom), but they are clearly significant if the information from the three experiments is pooled.

The correlations of type (A) are, as a rule, considered the best estimates. In these experiments, however, they are of little importance for investigations dealing with the percentage nitrogen. For the treatments are so definite that there is little variation between the percentage nitrogen figures determined for plots on the same treatment. These agree as a rule in their first and often in their second decimal. It is not surprising that differences of this order are found not to be correlated with other plant attributes. Even in case relations were found to exist for such differences, they would be of practical value only if the relationship could be proved to hold for a wider range of the percentage nitrogen figures. This necessitates a study of the correlations of type (B) which can be estimated with reasonable accuracy if the data from the three experiments are pooled in a "between experiments"—"within experiments"—analysis. The treatment averages may be regarded as a stratified sample which is representative of soil conditions produced by the experimental treatments.

Various experiments have been carried out to establish if possible correlations between the percentage nitrogen ($y$) and other plant attributes such as the 1000-corn weight ($x_2$) and the average number of surviving tillers per plant ($x_1$). An attempt will be made here to establish a *multiple* regression between these variates, i.e. an equation from which $y$ may be calculated if *both* $x_1$ and $x_2$ are known. Dr Hunter has shown that the time factor is most important in determining the nitrogen content, and this suggested the choice of these two plant attributes as the two independent variates $x_1$ and $x_2$. The first ($x_1$) is taken as a measure of high tillering and thus of high vegetative intensity during the *early* stages of plant development whilst an additional(!) increase of the 1000-corn weight ($x_2$) is taken as an indication of growth during the *later* stages of the lifetime of the plant (treatment *G*). The analysis is shown below, Table V A, the sums of squares and products having been calcu-

Table V A. *Test for the significance of the regression of nitrogen percentage on average number of surviving tillers and* 1000-*corn weight*

| | D.F. | Sum of squares | Mean square | |
|---|---|---|---|---|
| Total | 17 | 3·90561 | | |
| *Double regression* | 2 | 2·38656 | 1·19328 | *Sig. at* 1 % |
| *Residual* | 15 | 1·51905 | 0·10127 | |

lated from the "within experiments" variation of the treatment averages ($5+6+6=17$ degrees of freedom). The double regression is seen to be highly significant, the multiple correlation being given by

$$R=0{\cdot}782.$$

The regression is given by the equation

$$y=-1{\cdot}280-0{\cdot}284x_1+0{\cdot}083x_2. \qquad \ldots\ldots(1)$$

A similar equation is found to hold for *differences* between any two treatment averages from the same experiment, viz.

$$\Delta y=-0{\cdot}284\Delta x_1+0{\cdot}083\Delta x_2, \qquad \ldots\ldots(2)$$

where $\Delta y$, $\Delta x_1$ and $\Delta x_2$ are respectively the differences between the two respective values of percentage nitrogen, average number of surviving tillers per plant and 1000-corn weight. This latter equation does not involve the grand averages of the plant-attributes and its accuracy is determined by the standard errors of the two regression coefficients $b_1=-0{\cdot}284$ and $b_2=0{\cdot}083$ which are given by S.D. of $b_1=0{\cdot}070{,}9$, S.D. of $b_2=0{\cdot}028{,}1$. Although the above regression is applicable only to conditions similar to the experimental conditions described in the paper, a relationship of this kind may be taken as an explanation of certain observations recorded by Russell & Bishop (1933, p. 324), who found that among corns from the *same* sample the larger ones tended to have a higher percentage nitrogen than the smaller ones, whilst no such relationship could be found between corns of different sizes taken from *different* samples. Most likely corns from the same sample have been harvested from crops with the same average number of surviving tillers per plant, so that in equation (2) $\Delta x_1$ would be 0 and a positive partial correlation between $y$ and $x_2$ is seen to relate high 1000-corn weight with a high nitrogen percentage. Not so, however, for corns from different samples, as these might have been harvested from crops with different average numbers of surviving tillers per plant.

This explains the nature of the relationship between the three plant attributes. It would appear from these experiments that the 1000-corn weight ($x_2$) by itself cannot be taken as an index of the percentage nitrogen ($y$). Indeed, the ordinary correlation [of type (B)] between these two plant attributes is

$$r=0{\cdot}44,$$

and is insignificant (16 degrees of freedom). The question therefore arises whether the effect of 1000-corn weight in the above regression might not be insignificant, so that it would be sufficient to use the average number of surviving tillers per plant ($x_1$) as an index for the percentage nitrogen ($y$). [The correlation between $y$ and $x_1$ is $0{\cdot}640$ and is significant at 1% (Table IV A).]

This point, however, is easily decided by testing the significance of the two partial regressions $b_1$ and $b_2$. Both exceed their respective standard errors (stated above) to be returned as significant at the 1% level by the $t$-test.

Whilst it is not surprising that the partial regression, $b_1$, of percentage nitrogen ($y$) on average number of surviving tillers ($x_1$) is significant, it is remarkable that the same is true for $b_2$, the partial regression on 1000-corn weight ($x_2$). For we have just seen that the ordinary correlation between $y$ and $x_2$ is insignificant.

This shows that a knowledge of the 1000-corn weight supplements the relationship between nitrogen percentage and average tillers per plant just at the point where the latter fails to be satisfactory by indicating growth conditions after flowering, which in these experiments are characterized by a nitrogen metabolism.

This may be demonstrated by an inspection of Table III, p. 478, where a comparison of treatment $B$ with treatment $G$ on one hand and treatment $A$ with treatment $E$ on the other hand clearly confirms the point at issue.

Another relationship which is of particular interest with the varieties under consideration is that between yield and average number of surviving tillers per plant. In these experiments these two plant attributes are very highly correlated (see Table IV A). Yet, in order to investigate the role played by the corn weight a multiple regression will be considered, viz. a relation between average grain per plant ($Y$), average number of surviving tillers per plant ($x_1$) and 1000-corn weight ($x_2$).

The partial regression coefficients $B_1$ and $B_2$ denoting respectively the partial regressions of ($Y$) on ($x_1$) and ($x_2$) are given below together with their standard errors:

$$B_1 = 0{\cdot}885 \qquad B_2 = -0{\cdot}0113$$
$$\text{S.E. of } B_1 = 0{\cdot}105 \qquad \text{S.E. of } B_2 = 0{\cdot}0414.$$

Whilst $B_1$ is highly significant the value of $B_2$ is quite insignificant.

The result of the test clearly indicates that in these experiments yield is almost entirely determined by the number of surviving tillers per plant whilst 1000-corn weight is quite irrelevant.

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# ON THE EXCHANGE OF BULL SEMEN BETWEEN ENGLAND AND HOLLAND

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In the spring of 1937 an experiment was conducted to test existing commercial facilities for the transport of bull semen between England and Holland. Samples were sent to and received from Holland via Royal Dutch Air Lines, to whom the authors wish to express their sincere thanks for most useful co-operation. The minimum interval between collection in one country and insemination in the other was 28 hr. and the maximum, 57 hr.

The artificial vagina was used in collecting the semen (Walton, 1938). In England the bull mounted a non-oestrus cow held in a breeding crate (Edwards & Walton, 1938); in Holland a cow constantly in oestrus was used. The semen was examined, stored undiluted in small sterile glass tubes, and after standing for 1 hr. in water at 10° C. was dispatched in a thermos flask containing ice. In the beginning several flasks wrapped in corrugated cardboard arrived badly smashed, and an alternative method of packing was tried which consisted of a wooden box with rubber sponges cushioning the flask. In the end, however, secure and economic transport was effected by having a good quality flask with at least six layers of corrugated paper round it and plugs of the same materials in the ends.

## Results with semen sent from England to Holland

The bull (Nico's Lindberg) from which the collections were made is a pure-bred imported Friesian in the herd of Messrs H. J. and H. G. Martin, Littleport, Cambridgeshire. His service record in the herd prior to the collections had been excellent, and the quality of the ejaculates sent to Holland in the beginning were very satisfactory. Columns 4 and 5 in Table I give the relevant particulars. Ejaculates Nos. 5, 6, 7, 9 and 11 showed a decline in volume and density although No. 8 (1 June) was the best of all. (Sample 10 was from a Shorthorn bull and was sent with sample 9 because of the poor quality of the latter.)

In the absence of critical testing facilities in Holland the only criterion

of condition on arrival is that of comparative motility. There is no doubt that the densest samples arrived in best condition. Samples of only medium density, 300–500 million sperm per c.c., showed poor motility after storage.

The cows inseminated were in commercial dairy herds for which one of the authors (J. S.) is veterinary surgeon, and in which up to 800 cows are artificially inseminated annually. The semen was not diluted prior to insemination, and the total quantity was divided equally amongst the cows available. In gauging the results—eight cows have since calved normal live calves out of the twenty-six that were inseminated—it is advisable to consider the cows individually. Col. 6 gives the breeding record of each cow prior to insemination, col. 7 the result of the insemination. It is always difficult in these cases to apportion credit or discredit—to decide, for example, if a cow that has failed to conceive to three normal matings prior to the experimental insemination should be considered a test cow for the quality of the semen. The results with the best sample (No. 8) illustrate the case. With it six cows were inseminated and three became pregnant; of these two had not been bulled previously and one had been bulled once and inseminated once. Of the three cows which did not conceive, one (16) had not conceived to August of 1937, and another (18) had been bulled twice and inseminated once prior to the experimental insemination and afterwards was given ovarian treatment before conceiving in July. The third (21), bulled once and inseminated once prior to the experiment, conceived after two further heat periods.

No pregnancies resulted from sperm of moderate density (300–600 million per c.c.) arriving in poor condition. Among the samples giving positive results the time between collection and insemination was as follows:

| No. of sample | Age (hr.) | No. of pregnancies |
|---|---|---|
| 1 | 28½ | 2 |
| 10 | 31 | 1 |
| 4 | 46 | 2 |
| 8 | 46 | 2 |
| 8 | 57 | 1 |

There is no suggestion, of course, that 57 hr. is the maximum, for there was no test of a longer storage time. Conception with semen stored up to 4 days has been obtained at Cambridge.

Table 1

| 1 Sample No. | 2 Date sent | 3 Hours stored | 4 Vol. semen c.c. | 5 Remarks on semen (a) Density on despatch | (b) Motility on arrival* | 6 No. of cow | 7 Cow's service record prior to experimental insemination | 8 Result | 9 Remarks |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 30. iv. 37 | 28½ | 5 | Very good | Good | 1 | Bulled once | Pregnant | — |
| | | 28½ | | | | 2 | Not bulled | Pregnant | — |
| 2 | 4. v. 37 | 46 | 4¾ | Very good | Poor | 3 | Not bulled | Not pregnant | — |
| 3 | 14. v. 37 | 41½ | 4 | Very good | Very good | 4 | Not bulled | Not pregnant | Oestrum ended? 20 hr. after first signs and vagina dry |
| 4 | 18. v. 37 | 46 | 3¾ | Very good | Good | 5 | Inseminated once | Not pregnant | Inseminated once since; not pregnant |
| | | 46 | | | | 6 | Not bulled | Not pregnant | — |
| | | 46 | | | | 7 | Not bulled | Pregnant | — |
| | | 46 | | | | 8 | Not bulled | Not pregnant | Pregnant to insemination on 18 June |
| | | 46 | | | | 9 | Not bulled | Pregnant | — |
| | | 46 | | | | 10 | Not bulled | Not pregnant | Inseminated at very beginning of oestrum |
| 5 | 20. v. 37 | 33 | 2 + 2½ | Good | Poor | 11 | Not bulled | Not pregnant | Has uterine catarrh. Twice inseminated since—not pregnant |
| 6 | 25. v. 37 | 54 | 2½ | Very good | Poor | 12 | Inseminated once | Not pregnant | Pregnant to insemination on 17 June |
| | | 54 | | | | 13 | Not bulled | Not pregnant | — |
| 7 | 27. v. 37 | 45 | 2½ | Good | Poor | 14 | Not bulled | Not pregnant | — |
| 8 | 1. vi. 37 | 46 | 6½ | Excellent | Very good | 15 | Not bulled | Pregnant | — |
| | | 46 | | | | 16 | Not bulled | Not pregnant | Inseminated August; not pregnant |
| | | 46 | | | | 17 | Bulled twice | Pregnant | — |
| | | 46 | | | | 18 | Bulled twice and inseminated once | Not pregnant | Given ovarian treatment later and pregnant to insemination in July |
| | | 57 | | | | 19 | Not bulled | Not pregnant | — |
| | | 57 | | | | 20 | Not bulled | Pregnant | — |
| | | 57 | | | | 21 | Bulled once, inseminated once | Not pregnant | Inseminated twice later and pregnant to second |
| 9 | 3. vi. 37 | 31 | 1½ | Poor | Dead | — | — | — | — |
| 10 | 3. vi. 37 | 31 | 4 | Very good | Very good | 22 | Not bulled | Not pregnant | Inseminated twice later; not pregnant |
| | | 31 | | | | 23 | Not bulled | Pregnant | — |
| 11 | 15. vi. 37 | 46 | 2 | Good | Poor | 24 | Not bulled | Not pregnant | Previous calving twins and retained placenta. Not pregnant to subsequent service |
| | | 47 | | | | 25 | Not bulled | Not pregnant | Pregnant to insemination 20 July |
| | | 47 | | | | 26 | Not bulled | Not pregnant | Pregnant to insemination 6 July |

* Motility on despatch all very good.

Results with semen sent from Holland to England

Five samples from a Holland Friesian bull owned by one of us (J. S.) were received in England in January and February 1937. At this time the bull was producing inferior semen (1–1½ c.c. per ejaculate with a density of about 300 million), and his service record in Holland was below normal. The semen did not arrive in good condition. Only three samples were considered suitable for insemination—one arriving on 9 January and two on 11 February. The first was 72 hr. old, the second 48 hr. and the third 28 hr. In each case the semen was diluted 1 : 1, and 1 c.c. of the diluted semen inseminated. The following are the particulars of each insemination.

*Sample* (1). Inseminations on 9 January 1937. Semen 76 hr. old:

Cow No. 1. Served once previously. Normal insemination. Returned to service 22 February.

Cow No. 2. Not served since calving. Normal insemination; returned to service 12 January.

Cow No. 3. Served twice previously. Normal insemination. Returned to service in May.

*Sample* (2). Inseminations on 11 February 1937. Semen 48 hr. old:

Cow No. 4. Served twice previously. Cervix abnormally wide at time of insemination. Turned 21 April.

Cow No. 5. Not served since calving. Turned 9 March.

*Sample* (3). Inseminations 11 February 1937. Semen 28 hr. old:

Cow No. 6. Not served since calving. After insemination thought to be in-calf until June and then sold as not in-calf.

Cow No. 7. Not served since calving; normal insemination. Pregnant and calved 25 November 1937.

Cow No. 8. Not served since calving. Cow strained and pressed badly during insemination. Turned 22 February.

Again there is difficulty in assessing the results—one pregnancy from eight inseminations. It will be remembered that the quality of the semen was poor, and it is noteworthy that the one positive result was with semen only 28 hr. old. On the other hand, the cows, as the individual particulars reveal, probably had an influence on the results. For example, cow No. 2 returned to service 3 days after insemination; two cows had already failed to conceive to two normal services each, and two, although watched, were not seen to return to service after the insemination for 3–4 months.

## DISCUSSION

The transport of bull semen between England and the continent is a feasible proposition. The chief point to notice is that the sample of semen for transport must be as dense as possible—preferably not less than 1000 million sperm per c.c. Such a sample undoubtedly keeps its vitality best. (We have received an unpublished report of a very successful experiment with semen transported from Washington, U.S.A. to the Argentine. The semen arrived in very good condition, and after a storage interval of 7 days gave excellent results. The semen was collected by the rectal palpation method (Miller & Evans, 1934) and the very dense nature of sample collected in this way was probably responsible for the good storage quality). The density should always be measured by a sperm count, for it is not possible to judge it with reasonable accuracy either by appearance under the microscope or by its colour (opacity) in a glass tube.

It seems unnecessary to add that in an experiment of this kind or in any trial of artificial insemination only cows with normal breeding records should be used. It is difficult otherwise to judge the results. In the present case the desire to inseminate all cows in oestrum at the time of the arrival of the semen, together with the fact that in private pedigree herds there is a natural desire to reserve the best and normal cows to the herd bulls and utilize only the remaining ones for experimentation, were responsible for a group of experimental cows that left something to be desired.

The financing of the export and import is interesting; a nominal service fee of 10*s*. per ejaculate was paid in England, and the cost of packing and transport to Holland was about 4*s*. on each parcel. The total cost for the eleven samples was therefore £7. 14*s*. 0*d*., or just less than £1 per live calf. The expenses on sample 8, Table I, were 14*s*. for three live calves, or 4*s*. 8*d*. each. And of course more cows, had they been available, could have been inseminated with the good samples.

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# THE PRACTICAL APPLICATION OF AGE CONVERSION FACTORS TO DAIRY CATTLE PRODUCTION (BUTTERFAT) RECORDS

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MUCH work has been done and a great deal written on standardization of production records of dairy cattle to a strictly comparable basis. There appears, however, to be a lack of data demonstrating the practical results of such standardization methods under normal herd conditions such as would apply to the average breeder or commercial dairy farmer. It is neither intended in the scope of this study to cover the whole of the work carried out by other investigators into the calculation of conversion factors for age in dealing with comparisons of production records of dairy cattle at different ages, nor yet to suggest a further factor which will overcome *all* our present difficulties. Much remains to be done before the issue is sufficiently clear for mere summarizing. The use of age conversion factors is of very considerable importance at the present time, both to research workers and to the practical breeder, and with the advent of Sire Survey work (or Proven Bull work, as it is more familiarly known), the necessity for comparing production records at different ages has increased to such an extent that in many cases the value of the conclusions drawn is largely dependent on the accuracy of the adjusting factors used.

A number of adjusting factors such as effect of month of calving, and length of dry period, have been suggested by a number of investigators in making such comparisons, but the very real difficulty of completely covering all influences which cause environmental variation in production records has been repeatedly recognized, and most workers are now content to use only conversion factors for age, and to standardize the limit of length of lactations. Undoubtedly, given reasonable stability of herd conditions, these are the most important single considerations

in the comparison of records—particularly in the case of Sire Survey work. The narrowing down of adjusting factors to that for age alone involves a greater responsibility for the worker to ensure that the factor used will be strictly applicable under ordinary herd conditions. This does not appear to the authors to have been considered sufficiently in the evolution of a number of the age conversion factors at present in use.

Many of these, of American origin, have been calculated direct from Registry of Merit data and similar sources, where a large number of records in each age class can be secured. The assumption is no doubt valid that the use of such large numbers ensures a certain homogeneity in the productive class of animal in each age group, although it has been recognized that this assumption weakens as the classes increase in age. After arriving at the average production for each age class in this manner, the change in the level of production from age to age is stated to operate as a ratio, or addition of a percentage, when applied individually. This involves the assumption that the ratio form of conversion (that is, the addition of a percentage) holds good for all levels of production, but unfortunately the nature of the data used precludes any adequate test of this fundamental assumption. The alternative to the use of Registry of Merit, and similar data, is the use of lifetime records of production but it would seem that there is a definite lack of such data available.

Sanders (1928), calculates age conversion factors on the ratio basis, but draws attention to the fact that this does not operate with strict accuracy when applied to high and low levels of production. He suggests that the cause might well be nutritional, and on account of the relatively small number of animals included, and the complications "that would be introduced into an otherwise simple method of standardization", does not pursue the problem further.

Kay & M'Candlish (1929), in an analysis of Ayrshire records for animals with at least five consecutive lactations, calculate age conversion factors on the basis of population averages, but suggest that the ratio form of conversion factor arrived at should be further checked by determining its accuracy in application to low-producing and high-producing animals—or rather, to all levels of production. The authors have unfortunately been unable to find any further reference to the investigations of Kay & M'Candlish into age conversion factors, and are therefore unaware as to whether this problem has been further resolved by them.

Tuff (1931), from an analysis of seventy-four animals with seven consecutive lactation years, concludes that "the increase in milk yield

from young to full-grown age of an individual can neither be summarized by a constant addition nor by a percentage addition alone", and proceeds to state a formula of the nature of $Y = 1096 + 0{\cdot}447X$ for conversion from two years ($X$) to maturity ($Y$) for the first 180 days' milk production. There appears to be a growing dissatisfaction with the present form of conversion factors, and the University of Illinois (1933–4; 1934–5) have provided a further avenue for investigation by suggesting that "The expression of yield in terms of milk energy per unit of live weight would do away with troublesome and sometimes questionable correction factors". This hypothesis suggests that either mammary tissue increases with size, or capacity to produce increases with size. It provides no allowance for stimuli associated with growth. If correct, it does not explain why production falls off with advanced age, nor would it be suggested that the influence of senescence on production operates through a reduction in size. It is very probable that size plays some part in determining increasing production with advancing maturity, but it certainly cannot be said to cover the whole of the circumstances associated with fluctuating production according to age.

Obviously, then, considerable work remains to be done before age conversion factors can be used extensively to give reliable results under ordinary herd conditions. The authors feel, however, that insufficient attention has been paid to the question of the nature of age conversion factors. A broad study of lifetime records aimed at making corrections for all levels of production would automatically overcome the objection of the Illinois workers to the use of correction factors as such, and would at the same time meet objections which must occur with the use of conversion factors based on change in weight alone. In order to test this theory and to evolve conversion factors suitable under ordinary herd conditions in New Zealand, an analysis of lifetime records of production was made from data available in the files of the New Zealand Co-operative Herd Testing Association, an organization testing over 100,000 cows annually.

The area covered by the testing operations of the New Zealand Co-operative Herd Testing Association covers the whole of the Auckland Province (excluding the Bay of Plenty), and part of North Auckland.

All cows in this survey were of the Jersey type. In some cases (definitely a minority) the animals may have been of mixed breeding, but the Jersey strain predominated.

## Experimental

*Source of data.*[1] All herds which had tested for at least six consecutive years, and in which the cows could be clearly traced from year to year, were analysed on a strictly unselective basis. All such lifetime records of production commence from 2 years of age (2 years of age under these conditions means not older than 2 years 6 months at the time of first calving); all records were made on twice a day milking under normal herd conditions. It might here be mentioned that under normal conditions in the area from which these records were drawn, dairy cattle are grazed on mixed pastures during the whole of the year and practically no feeding of meals takes place. Under New Zealand Group Herd Testing conditions, all animals in the herd must be submitted to test, and the testing visits occur monthly as is customary in most overseas countries. Records for cows which were definitely abnormal (sick, aborted, etc.) at the time of first calving were discarded, but apart from this all reasonably normal lactations were included in order that the test should apply strictly, and unselectively, to average herd conditions.

*Technique.* Lactation periods in excess of 320 days were reduced to the first 320 days' production in order to achieve a more uniform comparison between all lactations; in those cases where an animal had no record in a particular season through not being in calf, etc., an average was substituted based on an average of the preceding and succeeding lactation. This method is, of course, open to objections, but it appears to the authors to be satisfactory in arriving at population averages according to age, and the average maturity values for individual cows. In no case, however, was an average used when the analysis depended on the individual use of the production for the season in question. The proportion of averaged lactations to normal lactations was approximately 3 per cent.

Each animal was tested in the same herd throughout its lifetime, so

[1] Group Herd Testing in New Zealand is the counterpart of Control Societies in Denmark, Recording Societies in Great Britain, and Dairy Herd Improvement Associations in U.S.A. The production records are recorded at 30-day intervals, and calculations are carried to a point midway between the visits of the Recording Officer.

The records are those of butterfat production, milk values being absent from the earlier data. It should be noted that in order to correct for increase in milk production it would be necessary to use the butterfat test (that is, percentage of milk fat), and to calculate the maturity milk production from the maturity fat production. This involves the assumption that there is little change in the percentage of milk fat according to age increase—an assumption supported by many analyses, and already accepted in age correction work by means of the percentage addition.

that relative stability of herd conditions, within certain limits, can be assumed. Changes in farm economy and technique would affect the extent of improvement from 2 years to maturity in certain herds, but as there were four series of lifetime records commencing in the years 1928, 1929, 1930 and 1931 respectively, it follows that any significant difference due to improved herd or farm conditions should have become apparent in the later comparisons. This variation would, in any case, not unduly affect the nature of the conversion factor, but would alter the amount of such factor.

The variation in seasonal production due to climatic conditions can be largely measured by the change in annual average for the whole of the testing districts from which these records were drawn. This annual average comprises between 90,000 and 120,000 cows, and although variation might be slightly influenced by changes in membership and by improved selection of dairy stock, over a period of only nine seasons and with such a large number of animals, it seems reasonable to assume (for purposes of smoothing) that the change in the annual average will provide the nearest approach to a measure of the extent to which seasonal production has been influenced by climatic conditions.

In the tables quoted, and also the statistical constants calculated, no adjustment has been made for seasonal conditions unless it is specifically so stated.

One difficulty in correctly interpreting age conversion factors lies in determining what shall be regarded as the correct maturity equivalent. When it is remembered that the major purpose of age conversion factors lies principally in the daughter-dam comparisons carried out for Sire Survey work, it seems reasonable to assume that in most cases the average production of the four lactations at 4, 5, 6, and 7 years of age would constitute a reasonable interpretation of the maturity equivalent.

## ANALYSIS OF DATA

The main question requiring an answer is—given an immature production record, what will be the maturity equivalent production? The nature of the problem is such that the answer can be expected to be true, at most "on the average". An analysis, on a regression basis, of the various correlation tables mentioned below, at once suggests itself as a way of arriving at some form of estimate of the maturity equivalent production. It cannot be too strongly emphasized that the validity of the use of any relationship thus arrived at, is based on the

assumption that the essential conditions are the same as those of the table on which it is based.

*Population statistics.* Tables I and II indicate the general average of production according to age. In studying the change in production according to age, the results quoted in Table I should be read in conjunction with those quoted in Table II as the truth probably exists somewhere between the two sets of values.

Table I. *Analysis of lactation production according to age*

| | | Average lactation production (lb. butterfat) according to age | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | No. of cows | 2 yr. | 3 yr. | 4 yr. | 5 yr. | 6 yr. | 7 yr. | 8 yr. | 9 yr. |
| A | 58 | 257 | 310 | 334 | 337 | 366 | 367 | 342 | 336 |
| | Index No. | 70 | 84 | 91 | 92 | 100 | 100 | 93 | 92 |
| B | 161 | 260 | 297 | 317 | 338 | 363 | 336 | 326 | |
| | Index No. | 72 | 82 | 87 | 93 | 100 | 93 | 90 | |
| C | 483 | 242 | 281 | 328 | 338 | 343 | 331 | | |
| | Index No. | 71 | 82 | 96 | 99 | 100 | 97 | | |
| Total | 702 | 247 | 287 | 326 | 338 | 349 | 335 | | |
| | Index No. | 71 | 82 | 93 | 97 | 100 | 96 | | |

Table II. *Analysis of lactation production according to age (smoothed to eliminate seasonal variations)*

| | | Average lactation production (lb. butterfat) according to age | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Group | No. of cows | 2 yr. | 3 yr. | 4 yr. | 5 yr. | 6 yr. | 7 yr. | 8 yr. | 9 yr. |
| A | 58 | 257 | 290 | 335 | 355 | 354 | 346 | 336 | 316 |
| | Index No. | 72 | 82 | 94 | 100 | 100 | 97 | 95 | 89 |
| B | 161 | 247 | 293 | 331 | 334 | 344 | 327 | 308 | |
| | Index No. | 72 | 85 | 96 | 97 | 100 | 95 | 90 | |
| C | 483 | 246 | 281 | 318 | 327 | 326 | 309 | | |
| | Index No. | 75 | 86 | 97 | 100 | 100 | 94 | | |
| Total | 702 | 247 | 284 | 322 | 331 | 332 | 316 | | |
| | Index No. | 74 | 86 | 97 | 100 | 100 | 95 | | |

*Conversion from 2 years to maturity.* From a correlation surface for the yield of 702 cows tested from 2 to 7 years, the relationship between 2-year-old production and mature yield, i.e. the average of 4, 5, 6 and 7 lactations was studied. The statistical parameters are as shown below (Table III).

Table III

| | Mean (lb. fat) | Variance* | Covariance* |
|---|---|---|---|
| 2-year-old production ($Y$) | 246·8 | 8·62 | 5·92 |
| Maturity equivalent ($X$) | 336·8 | 9·83 | |

$$r^2 = 0{\cdot}4139 \quad \text{so} \quad r = +0{\cdot}64.$$

Regression equation $X = 167{\cdot}2 + 0{\cdot}6873\,Y$,

where $X$ is estimated maturity equivalent and $Y$ is the 2-year-old production.

* The variances, covariances, and sums/squares throughout are in class interval units².

The analysis of the sums of squares corresponding to the rows of the table, i.e. to the $X$ variate, gives:

| Source of variation | | Sums/squares* ($X$) | | Degrees of freedom | | Mean variance |
|---|---|---|---|---|---|---|
| Linear regression | Between rows | 2857·24 | 3016·51 | 1 | 17 | |
| Deviations from linear regression | | 159·27 | | 16 | | 9·95 |
| Residual within rows | | | 3886·54 | 684 | | 5·68 |
| | Total | | 6903·05 | 701 | | |

A comparison of the two variances shown, by means of Fisher's $z$ test, leads to value $z=0{\cdot}2803$. This lies between the 1 and 5% point, being just above the 5% point, so the deviations from linear regression are barely statistically significant.

A similar analysis of the four groups comprising this table, those animals coming in as 2-year-olds in the 1928–9, 1929–30, 1930–1, and 1931–2 seasons, give values of $z$ between the 1 and 5% points for the 1929–30, 1931–2 groups, while the values of $z$ for the remaining two seasons are not significant at the 5% point.

*Conversion from* 3 *years to maturity.* The previous calculations were repeated in order to study the relationship between 3-year-old production and mature yield. The statistical parameters are shown below (Table IV).

Table IV

| | Mean (lb. fat) | Variance | Covariance |
|---|---|---|---|
| 3-year-old production ($Y$) | 286·5 | 10·86 | 6·72 |
| Maturity equivalent ($X$) | 337·2 | 9·59 | |

$r^2=0{\cdot}4330$, so $r=+0{\cdot}66$

Regression equation $X=160+0{\cdot}6184\,Y$,

where $X$ is estimated maturity equivalent and $Y$ is the 3-year-old production.

| Source of variation | | Sums/squares ($X$) | | Degrees of freedom | | Mean variance |
|---|---|---|---|---|---|---|
| Linear regression | Between rows | 2641·01 | 2773·18 | 1 | 20 | |
| Deviations from linear regression | | 132·17 | | 19 | | 6·96 |
| Residual within rows | | | 3326·15 | 615 | | 5·41 |
| | Total | | 6099·33 | 635 | | |

A comparison of the mean variances gives a value of $z$ which is not statistically significant at the 5% point.

*Check on use of the average of* 4, 5, 6 *and* 7 *years old lactations as "maturity equivalents".* As a further check on the validity of the conclusions drawn by assuming that the average of the 4, 5, 6 and 7 years old lactations constitutes a reliable basis for the maturity equivalent, the records of 940 cows tested for five consecutive seasons under similar

herd conditions, were analysed and the highest production up to and including the fifth lactation used as the basis of the "mature equivalent". This method eliminates to a large extent the inclusion in the maturity equivalent of productions which may have been adversely affected by the various environmental causes. (The use of five consecutive lactations instead of six enabled the authors to utilize a greater number of records—it was considered that while the actual equation might not give as accurate a conversion as the six consecutive lactation analysis, it would be quite an adequate check on the *nature* of the conversion, and this, after all, is the important consideration at the present time.)

The comparison of the 2-year-old production with the highest production up to and including the fifth lactation, yields the following results (No. of cows = 940).

Table V

| | Mean (lb. fat) | Variance | Covariance |
|---|---|---|---|
| 2-year-old production ($Y$) | 244·9 | 9·16 | 7·26 |
| Highest production ($X$) | 369·6 | 13·11 | |

$r^2 = 0{\cdot}4394$, so $r = +0{\cdot}66$.

Regression equation $X = 175{\cdot}3 + 0{\cdot}7932\,Y$.

| Source of variation | | Sums/squares ($X$) | | Degrees of freedom | | Mean variance |
|---|---|---|---|---|---|---|
| Linear regression | Between rows | 5415·98 | 5662·47 | 1 | 18 | |
| Deviations from linear regression | | 246·49 | | 17 | | 14·50 |
| Residual within rows | | | 6661·47 | 921 | | 7·23 |
| | Total | | 12323·94 | 939 | | |

The value of $z$ for the comparison of the variances shown lies between the 1 and 5% points.

It will be noted that the equation is similar in form and differs only in degree from those obtained from Tables III and IV, thereby justifying the "maturity equivalent" basis used for the purpose of studying the *nature* of conversion factors for age.

*Check on 2-year-old production by use of C.O.R. data.* With both the previous methods no adequate check is secured on the nature of the 2-year-old production, and resort was therefore made to the use of the Government Official C.O.R.[1] records for the Jersey breed. The method

[1] C.O.R. or Certificate of Record testing is carried out by the New Zealand Department of Agriculture and includes only selected registered pure-bred dairy cows. The milk is weighed daily by the herd owner, and tested monthly by an officer of the Department of Agriculture, whose visit to the herd covers 2 days, thereby ensuring complete stripping prior to testing. The records in the above table refer to the 365 day division of C.O.R. testing.

adopted was to select all cows which had been tested under this system at 2 years of age (not exceeding 2 years 6 months at commencement of first test) and to compare this record with the re-entry record in the same herd at 4 years of age or later. Under the C.O.R. system of testing it can be safely assumed that the animal is tested and fed under the best possible conditions pertaining in that herd, and that the record includes normal calving and good test conditions throughout the lactation.

The analysis of the C.O.R. data, production at 2 years of age compared with the re-entry record in the same herd at 4 years of age or later, gives the following results (No. of cows = 182):

| | Mean (lb. fat) | Variance | Covariance |
|---|---|---|---|
| 2-year-old production ($Y$) | 419·2 | 19·02 | 13·40 |
| Re-entry record ($X$) | 550·3 | 30·34 | |

$r^2 = 0{\cdot}3109$, so $r = +0{\cdot}56$.

Regression equation $X = 255{\cdot}0 + 0{\cdot}7044\,Y$.

| Source of variation | | Sums/squares ($X$) | | Degrees of freedom | | Mean variance |
|---|---|---|---|---|---|---|
| Linear regression | Between rows | 1717·23 | 2343·60 | 1 | 21 | |
| Deviations from linear regression | Between rows | 626·37 | | 20 | | 31·32 |
| Residual within rows | | | 3178·38 | | 160 | 19·86 |
| Total | | | 5521·98 | | 181 | |

The value of $z$ for the comparison of the two variances shown is not statistically significant at the 5% point. A point worthy of note is the large residual variance for the C.O.R. data. It is nearly four times that for Table III, hence the standard error of an estimate of mature production, on the basis of the C.O.R. data, is approximately twice that for the herd testing data secured under ordinary herd conditions.

This is not unexpected in view of the wide range of efficiency in preparing stock for submission to the C.O.R. test. Differences in conditions of feeding, management, etc., would play a much greater part in the variation between records than would be noticeable under ordinary herd conditions.

*Increase in production from 2 years to maturity under reasonably stable and good herd conditions.* A further criticism which may be directed against the data in Tables III and V is that cows which commenced their test as 2-year-olds in low-producing herds would tend to improve more compared with their later productions, than animals commencing their test in high-producing herds. The production of the former animals would in some cases include natural improvement, plus improvement due to increasing farm economy and efficiency, whereas the latter would

include mainly only natural improvement with age-herd conditions being fairly stable during their lifetime.

In order to meet this objection a table was compiled of all animals with six consecutive lactations (commencing at 2 years of age) which were tested in herds which commenced with, and maintained an average of at least 280 lb. fat throughout the period under review. (The average production for all herds included in the survey would be approximately 250 lb. fat—averages computed on the basis of all cows in milk 100 days or more.)

From this data we obtain the following results (No. of cows 170):

| | Mean (lb. fat) | Variance | Covariance |
|---|---|---|---|
| *2-year-old production* ($Y$) | 297·0 | 6·20 | 3·65 |
| Maturity equivalent ($X$) | 379·0 | 7·56 | |

$$r^2 = 0{\cdot}2846, \quad \text{so} \quad r = +0{\cdot}52.$$

$$\text{Regression equation } X = 203{\cdot}9 + 0{\cdot}5894Y.$$

| Source of variation | | Sums/squares ($X$) | | Degrees of freedom | | Mean variance |
|---|---|---|---|---|---|---|
| Linear regression | Between rows | 365·88 | 513·51 | 1 | 14 | |
| Deviations from linear regression | Between rows | 147·63 | | 13 | | 11·35 |
| Residual within rows | | | 772·00 | 155 | | 4·98 |
| | Total | | 1285·51 | 169 | | |

On comparing the two mean variances we find the value of $z$ is between the 1 and 5% points.

In order thoroughly to test the operation of the two conflicting age correction factors, all 2-year-old cows in Table III were corrected to maturity on the basis of a percentage addition and the regression formula. These values were calculated direct from Table III, the percentage addition being $\frac{336{\cdot}8 - 246{\cdot}8}{246{\cdot}8} = 0{\cdot}365$ (or $X = 1{\cdot}365Y$), and the regression $X = 167 + 0{\cdot}687Y$ where $X$ = the maturity equivalent and $Y$ the 2-year production. The deviations of the corrected values from the actual maturity equivalent were then arrayed in 20 lb. fat intervals (from zero) on either side of the 2-year production class, according as to whether the production had been under or over-corrected. All deviations of more than 140 lb. fat were treated as deviations of between 141 and 160 lb. fat—this is valid for purposes of comparison only, and obviously favours the percentage correction as vide Table VI. As will be seen from the total sum of squares, not only is there a very definite bias in the percentage correction, but the correction by means of the regression formula gives better results throughout the table.

Table VI. *Comparison of variances from actual mature yields, of records corrected to maturity by (a) percentage addition and by (b) regression coefficient*

| 2-year production class (butterfat) | Percentage correction* | | | Regression correction† | | |
|---|---|---|---|---|---|---|
| | Cows under-corrected | Cows over corrected | Total S/S | Cows under-corrected | Cows over-corrected | Total S/S |
| 81–100 | 77·1 | — | 77·1 | 12·4 | 0·3 | 12·7 |
| 101–120 | 86·2 | — | 86·2 | 0·6 | 9·0 | 9·6 |
| 121–140 | 319·4 | — | 319·4 | 72·6 | 39·4 | 112·0 |
| 141–160 | 197·0 | — | 197·0 | 30·3 | 31·6 | 61·9 |
| 161–180 | 444·0 | 54·7 | 498·7 | 177·7 | 258·9 | 436·6 |
| 181–200 | 557·5 | 28·0 | 585·5 | 214·4 | 155·8 | 370·2 |
| 201–220 | 446·6 | 34·8 | 481·4 | 210·5 | 121·5 | 332·0 |
| 221–240 | 313·1 | 100·8 | 413·9 | 200·4 | 132·4 | 332·8 |
| 241–260 | 262·3 | 194·3 | 456·6 | 272·4 | 162·0 | 434·4 |
| 261–280 | 143·1 | 537·3 | 680·4 | 210·4 | 345·6 | 556·0 |
| 281–300 | 77·0 | 474·5 | 551·5 | 246·3 | 192·5 | 438·8 |
| 301–320 | 47·7 | 799·3 | 847·0 | 194·5 | 299·6 | 494·1 |
| 321–340 | 0·6 | 280·7 | 281·3 | 72·3 | 35·6 | 107·9 |
| 341–360 | — | 267·0 | 267·0 | 10·1 | 33·0 | 43·1 |
| 361–380 | — | 320·8 | 320·8 | 2·3 | 48·8 | 51·1 |
| 381–400 | — | 120·3 | 120·3 | 19·1 | 26·9 | 46·0 |
| 401–420 | — | 69·8 | 69·8 | 27·4 | — | 27·4 |
| 421–440 | — | 18·8 | 18·8 | 18·8 | — | 18·8 |
| | 2971·6 | 3301·1 | 6272·7 | 1992·5 | 1892·9 | 3885·4 |

* Percentage correction $X = 1{\cdot}365\,Y$. † Regression correction $X = 0{\cdot}687\,Y + 167$.

*Note.* "Cows under-corrected" means those cows whose actual maturity production exceeds the corrected 2-year-old production. "Cows over-corrected" means those whose actual maturity production is less than the corrected 2-year-old production.

## DISCUSSION

The above results seem to point quite definitely to the relationship between immature and mature production being of the nature of a *regression*. And further, a straight line regression appears to be quite adequate to represent the relation over the range of productions considered. (It is not asserted that the regression lines obtained from the foregoing tables are necessarily the "best" for the purpose of estimating mature production. These would have to be calculated according to the definition of "maturity production" and this will differ according to the nature of the problem encountered.)

Objection to the use of complicated or difficult formulae in correcting immature productions to a maturity basis has been soundly and repeatedly made. This objection, however, is distinctly invalid if the use of simple formulae gives unreliable results under normal herd conditions. To adjust formulae of the nature quoted in these tables to a basis giving reasonable

ease of application in practice does not present any considerable difficulty; for instance a formula of the nature $X = 0{\cdot}75Y + 160$, where $X$ is the estimated maturity production and $Y$ is the 2-year-old production, would appear to give substantially reliable results. This formula involves the calculation of three-quarters of the 2-year-old production plus 160 lb. fat in order to arrive at the estimated mature production.

Similarly $X = 0{\cdot}70Y + 150$ is a reasonable fit to the 3-year-old maturity production data and involves the addition of 150 lb. fat to seven-tenths of the 3-year-old production in order to obtain the estimated maturity production.

These are merely suggestions to demonstrate that the regression formulae quoted can be made fairly simple in practical application. In any case the difficulty of application cannot by any means outweigh the error involved in the use of a percentage addition in arriving at maturity equivalents.

An examination of the variances of the individual rows of Table III shows that the variances for 2-year-old animals of low production tend to be slightly larger than the average within array variance, and those for the higher producers, lower. This indicates the possibility of the environmental effect noted above, but the amount appears to be slight.

Although the scope of this investigational work covers the four breeds, Jersey, Friesian, Ayrshire and Shorthorn, only the results secured from the Jersey breed are analysed in this paper. As sufficient lifetime records of production accumulate for the other breeds further analysis will be made; at present the preliminary findings indicate that the position is much the same as with the Jersey breed, with necessary qualifications concerning the exact value of the conversion relation and the difference in rate of maturity.

The authors are fully aware that various other causes might give rise to considerable variation in the 2-year-old production, but the fact still remains that where the 2-year-old production has to be converted to a maturity basis, this must take place on the basis of the actual production figures available, and not on records adjusted in the light of later events, or theoretical considerations. Adjustments can of course be made for month of calving, length of service period, etc., but these are of minor significance compared with the conversion for age.

Also it is fully realized that there still exists the moot point as to what constitutes the "best" maturity equivalent. This, however, is a problem local in nature (in so far as it applies to the worker concerned), and, as previously pointed out, does not materially affect the present

discussion which seeks primarily to define the nature of conversion factors for age.

It is very possible that better methods of feeding and management according to prodnction level may correct the nature of conversion factors to something approaching the addition of a constant, but if conversion factors are to be applied under normal herd conditions, it appears that the addition of a proportion to the 2-year-old production is not only unjustifiable, but heavily penalizes the low-producing 2-year-olds and gives a considerable premium to the high-producing 2-year-olds.

This automatically brings about an entirely misleading position when herd sires are surveyed on the results of the daughters' early productions (which must inevitably tend to be the case if sires are to be proved at as early an age as possible), particularly when the productions in question are at all considerably removed from the general average.

The authors wish to make it quite clear that this paper is intended as a discussion of the relationship in practice between immature productions and maturity equivalents. They fully recognize that a number of influences affect the increase in production from early to mature age, but where a single correction factor for age is to be used, it is obvious that only an approximation to the truth can be obtained by the use of any formula, and this approximation must recognize as many as possible of the important influences.

The present paper suggests that insufficient attention has hitherto been directed to the operation, under normal herd conditions, of such age correction factors. Without discussing this particular phase of the problem in any great detail, it might be suggested that many influences occur in practice by which cows which were low producers at 2 years of age would show a considerable improvement at later ages, whereas it is obviously difficult for high-producing 2-year-old cows to maintain over several lactations a production considerably above their high 2-year-old production. It might further be argued that the addition of a percentage as an age correction factor assumes that the 2-year-old production is in perfect relationship with the normal producing ability of the cow, whereas this is highly improbable in actual practice, as shown by the correlation between different lactations of the same cow. Finally, the authors are unable to find any satisfactory evidence, based on an adequate number of lifetime records, which would indicate that percentage corrections, when calculated from total population values, are equally applicable to high- and low-producing cows within that population.

## SUMMARY

1. From an analysis of the records from 702 cattle of predominating Jersey type tested for at least six consecutive years under normal and average herd conditions in New Zealand, the authors have attempted a detailed study of the nature of the increase in production according to age.

2. They are unable to find any evidence supporting the theory that increase in production for dairy cattle operates as a percentage addition from early age to maturity.

3. The evidence secured from an analysis of 702 Jersey records of six consecutive lactations commencing at 2 years of age, strongly suggests that the increase in production according to age can be summarized neither by a percentage addition nor a constant addition alone, but is best represented by a regression formula of the nature $X = aY + b$, where $X$ equals the maturity production and $Y$ the immature production.

4. The strict regression formula will probably change according to the interpretation of "maturity equivalent", but in general its application should lose little of the simplicity of the ratio form of conversion.


In conclusion the authors wish to express their thanks to Professor W. Riddet, Massey Agricultural College, for valuable criticism and advice.

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# A STUDY OF THE DEVELOPMENT OF THE CHARACTERS OF THE FLEECE DURING GROWTH IN THE DIFFERENT REGIONS OF THE BODY

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(With Plates III–VI and Five Text-figures)

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## Introduction

It is not our intention to give a complete account of the literature on the subject, but we shall refer simply to those papers which seem to have a direct bearing upon the facts observed.

Duerden & Ritchie (1924) studied the development of the fleece in the South African Merino. Wildman (1932) made a very complete study of the coat of the foetus in several breeds, and he concludes that there are no differences in the mode of development. We must note, however, that this investigation is not based upon the real age of the foetuses but upon the relative lengths of the individuals examined. He mentions differences

found in foetuses that were twins and therefore of the same age. Galpin (1935) concluded from an examination of the coat of the foetus that the follicles of the fibres of the various regions develop at different times and that each region is a local determiner of its own development; she also suggests that "depression" in the potentiality in growth of the fibre is a local phenomenon. Iljin (1926) found that in the Himalayan rabbit each region of the body reacted differently to low temperatures, allowing recessive colour characters to appear in the hair covering the region concerned. He also recorded (1930) similar findings for the Siamese cat. The same author (1936) found that well-defined regions of the skin react differently, as regards the shedding of the fibres, to doses of thallium acetate injected; he concluded that different centres of keratinization exist in the body. Thomasset (1936), in a study of the variations in the fibres of wool grown during the course of a year on the shoulder and on the thigh, observed that the variations do not exhibit the same intensity everywhere—which seems to indicate a certain independence of these regions in the mode of reaction to environmental agents, a fact which is in agreement with the idea of the existence of "centres of keratinization" acting with a certain degree of independence. The same author (1937), studying the inheritance of the fleece characters in a Romney-Lincoln cross, arrived at the view that the inheritance of the fleece characters differ according to the regions of the skin, and that the way in which these characters are manifested may depend upon the late or early development of the region in question.

One must also note the differences of opinion among certain writers as to the distribution of the qualities of the fleece in different regions of the body. These differences of opinion are very probably due to real differences in the way in which the qualities of the fleece are distributed throughout the body. In breeds derived from recent crosses or crosses not yet fixed, e.g. the Corriedale and certain hybrid sheep, these differences are very pronounced from one region to another. Any investigation tending to elucidate the reason for these differences from one region to another from the physiological and genetic standpoint should, we believe, throw some light upon the interpretation of the relation that exists between the phenotype and the genotype of the animal. From the practical standpoint these investigations are obviously of interest, since they help to elucidate the problem of the homogeneity of the fleece and the problem of sampling to estimate the value of the fleeces of animals for breeding.

The points studied in this investigation are: (1) which of the four regions of the skin—the shoulder, the leg, the belly and the tail—exhibits

the earliest development and which the latest; (2) the relation existing between the relative earliness of the development in these regions and the nature of the fibres which develop upon them; and (3) which is the best suited region in a given stage of development at which to judge the qualities of the fleece.

### MATERIAL AND METHODS

The subjects studied were two twin lambs (shown at different stages of development in Pl. III, figs. 1*a–d*, 2*a–d*, born at the Animal Research Station, Cambridge, from a Suffolk ♂ × Suffolk (Border Leicester-Cheviot) ♀ cross.

One of these two lambs, No. 55, Pl. III, fig. 1, was completely covered, except for a small area over the shoulder, with long black hair at birth, and slowly lost this as it grew older. The other (No. 54, Pl. III, fig. 2) was shorter in fleece and not so dark in colour, and became white much more quickly as it grew older. Samples of wool were taken from these lambs every two months by shearing close to the skin from the following regions: shoulder, leg, belly and tail.

The samples were washed by immersing them for about 10 min. in two successive baths of xylol, and were then mounted with Canada balsam, and microscopically examined by transmitted light at a magnification of 540 diameters. The fibres were subsequently measured with an ocular micrometer on which 7·5 divisions $= 10\mu$. While measuring the fibres those which had any peculiarities such as medulla, colours, etc., and the degree of the peculiarity (medulla, more or less thick, colours of a greater or lesser intensity) were noted. As regards the shades of the coloured fibres, an estimation was made by eye according to an arbitrary scale ranging from white to deep black.

A macroscopic examination was made of the different types of fibres (especially in the tail region) also, by sorting them out with special forceps on a black or white background, according to the colour necessitated by the colour of the fibres.

### I. THE RELATIVE DEVELOPMENT OF THE FLEECE IN THE DIFFERENT REGIONS OF THE SKIN

The Suffolk is a breed in which the lambs are black at birth and the colour of the fleece changes to white as the lamb grows up. Examination of Pl. IV*a*, *b* and *c*, showing respectively the shoulder, leg and belly of lamb No. 54 and of Pl. V*a*, *b* and *c*, showing the shoulder, leg and belly

of lamb No. 55, show very clearly that as regards earliness of development one can class the regions observed in the following order: shoulder—leg—belly. The same conclusion can be drawn from an examination of Pl. III, figs. 1*a–d*, 2*a–d*. The tail shown in Pl. VI comes after the belly and is *the latest region of the body*.

*The criterion used for judging* the relative earliness of a region is the *quantity of coarse* and coloured hairs of the birthcoat found in the region examined, and the rapidity with which these disappear.

Since these types of hairs are present in the fleece of the young animals and disappear when the animal has reached the adult stage, one may conclude that they are characteristic of definite stages of growth and that the sooner they disappear from a region the sooner has this region passed through a given stage of development, hence the "earlier" is this region.

The shoulder of lamb No. 54 at 1 month old, Pl. IV*a*, has black coarse hairs at the end of the staple, but they extend over a little less than half the length, the rest being white. On the other hand, in lamb No. 55, Pl. V*a*, at 1 month old and at the same site the white portion of the staple takes up only the lower quarter approximately; a clearly black portion takes up approximately the upper half, and between the part that is clearly white and the black part there is a portion of intermediate colour composed either of fibres slightly brown in colour or of a mixture of coloured fibres with white ones, or the white fibres are much more numerous than at the end of the staple. This means that the shoulder in the two lambs produces at a certain period of its development fibres very similar in composition and in the way they are mixed, but that one of them (No. 54) subsequently begins to produce white fibres, or less coloured ones, in greater abundance. The shoulder of lamb No. 54 begins to produce white fibres abundantly at an earlier stage of development than that of lamb No. 55, or else the shoulder of No. 55 begins to produce black hairs before that of lamb No. 54, while beginning to produce white fibres at about the same time. Although the facts given here are not of the kind to decide the question, it is very interesting to observe, Pls. IV*a* and V*a*, that in the staple severed at 1 month old (25 January 1936) the black or coloured portion has a much greater absolute length in lamb No. 55 than in No. 54, whilst the white portions are of approximately the same length. The fact is very clearly seen in the other regions and the principle also applies in general when the fibres are studied individually. In the legs and the belly the difference in the length of the black part in lambs Nos. 55 and 54 is remarkable. It is important to note that the later a region is in development, the greater is this difference between the

length and the percentage of coarse and coloured fibres in a good (No. 54) and an inferior (No. 55) lamb, especially at the later stages of development.

An improved animal would therefore be one in which (1) the latest developing parts of the fleece would have the least possible undesirable fibres at the earliest possible age, even during the embryonic stage; and (2) the undesirable fibres would begin to appear in the embryo as late as possible. Apparently when the undesirable fibres begin to develop early they remain longer on the region. Since ontogenetic development is a repetition of phylogenetic development, in the light of what we have just seen, one may state that the breeds of sheep begin their improvement by changes in the earliest developing region (the shoulder) and will finish it by changes in the latest developing region (the tail). This agrees with the observation that can be made in studying the difference between the qualities of the wool of different regions in the ordinary animals of a given breed, and comparing them with highly improved animals of the same breed. In the ordinary animals one finds in the areas characterized by early development (shoulder, back) types of wool of which the quality sometimes exceeds the wool of highly improved animals of the breed; on the other hand in the late developing parts (legs) the qualities of the wool improve much more slowly, and wide differences are found between the highly improved and the ordinary animals.

## II. THE DEVELOPMENT OF CERTAIN WOOL CHARACTERS IN THE DIFFERENT REGIONS

### (a) *The colour of the fibres*

(1) *The intensity of the colour.* The intensity of the colour ranges from fibres very slightly coloured here and there by faint brown spots or stripes to fibres so dark that the light cannot pass through them at all when they are examined by a microscope with transmitted light, and in spite of the relatively great degree of fineness of certain fibres they are seen as completely opaque. We have arbitrarily classed the shades of the fibres into six kinds according to a scale drawn up by us namely: white fibres, slightly brown, brown, dark brown, black, and deep black.

(2) *The distribution of the shades along the length of the fibre.* One finds fibres that are entirely white, fibres entirely coloured, and numerous fibres quite black in the upper portion and quite white in the lower portion, showing that the same follicles can produce both black and white fibres. Sometimes in fibres that are coloured and constricted at some place one finds the constricted portion is white and the rest is black. The

black in this case passes into white after having passed successively through a series of colours which become lighter in shade as the fibre becomes gradually thinner.

Hence there are several sorts of follicles, (1) those which can produce only white fibres even before birth, (2) those which produce black fibres even a long time after birth, (3) those which produce black fibres before birth or during very active periods of growth and then when these periods are over produce white fibres constantly, (4) those which can produce alternatively white fibres or coloured ones of different shades.

(3) *Distribution of the various shades in the staple and in the different regions of the skin.* Tables I and II give in a summarized form an idea of how the colours are distributed in relation to each of the grades of fineness of the fibres, and the average fineness of each of the shades. These tables also show the percentage of fibres of different colours present at different ages and in the different regions. It will be seen from these tables that the fleece as a whole loses its dark colour first of all and primarily owing to a decrease in the coloured fibres in general and an increase in the white fibres and subsequently owing to a reduction in the ratio of strongly coloured fibres to weakly coloured ones, which may correspond to variations in the concentration in the same follicle (second case) and to the loss of coloured hairs with age (first case). The follicles with white fibres are really incapable of acquiring pigment whatever the conditions of growth may be, once the follicle is completely formed. In the portion of the fibre which has been formed while the follicle has not yet completed its development, i.e. while the point of the fibre has not yet been pushed out, brown colourations are produced in the fibres, even in those which later are entirely white—a fact which we observed in studying the tapered portion of the end of the fibres. But this type of follicle, once formed, loses completely its capacity to produce pigmented fibres. It is by the relative increase in the number of these follicles in proportion to the coloured ones or by some other important change that the modifying gene takes effect during the course of development. It is probable that it acts upon the coloured fibres (suppression or modification), and not by increasing the number of white fibres. The fact that the point of the white fibres is coloured to the very tip indicates that though these fibres are composed of cells which under normal conditions do not become pigmented, they are, nevertheless, not thus characterized at the time when they are dividing very actively during the formation of the follicle. The genes or factors which control the degree of colouration of the fibres might be, at

Table I. *Lamb No.* 54. *Distribution in diameter* ($7 \cdot 5 = 10\mu$) *of different coloured fibres in the fleece in different body regions and at different ages*

| Body region | Date | Colour of fibre | Fibres Total | Fibres % | No. of fibres with diameter of −8 | −13 | −18 | 23 | 28 | 33 | 38 | 43 | 48 | 53 | 58 | 63 | Av. fibre diameter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shoulder | 25. i. 36 | White | 365 | 92 | 5 | 128 | 206 | 26 | . | . | . | . | . | . | . | . | 17 |
| | | Brown (1) | 28 | 7 | . | 24 | 4 | . | . | . | . | . | . | . | . | . | 14 |
| | | Black (2) | 4 | 1 | . | 2 | 2 | . | . | . | . | . | . | . | . | . | 16 |
| | 21. iii. 36 | White | 392 | 95 | 1 | 64 | 255 | 69 | 3 | . | . | . | . | . | . | . | 18 |
| | | Brown | 19 | 4 | 1 | 11 | 7 | . | . | . | . | . | . | . | . | . | 15 |
| | | Black | 3 | 1 | 1 | 1 | 1 | . | . | . | . | . | . | . | . | . | 13 |
| | 16. v 36 | White | 387 | 96 | 1 | 45 | 252 | 84 | 5 | . | . | . | . | . | . | . | 19 |
| | | Brown | 12 | 3 | . | 5 | 6 | 1 | . | . | . | . | . | . | . | . | 16 |
| | | Black | 2 | 1 | . | 2 | . | . | . | . | . | . | . | . | . | . | 13 |
| Leg | 25 i. 36 | White | 349 | 87 | 2 | 65 | 159 | 109 | 13 | 1 | . | . | . | . | . | . | 19 |
| | | Brown | 44 | 11 | . | 27 | 15 | 2 | . | . | . | . | . | . | . | . | 15 |
| | | Black | 7 | 2 | . | 4 | 3 | . | . | . | . | . | . | . | . | . | 15 |
| | 21. iii. 36 | White | 377 | 94 | 1 | 8 | 83 | 168 | 105 | 11 | 1 | . | . | . | . | . | 23 |
| | | Brown | 12 | 3 | 1 | 4 | 6 | 1 | . | . | . | . | . | . | . | . | 16 |
| | | Black | 11 | 3 | . | . | 6 | 5 | . | . | . | . | . | . | . | . | 20 |
| | 16. v. 36 | White | 388 | 98 | . | 8 | 89 | 219 | 60 | 12 | . | . | . | . | . | . | 23 |
| | | Brown | 5 | 1 | . | 3 | 2 | . | . | . | . | . | . | . | . | . | 15 |
| | | Black | 3 | 1 | . | . | 3 | . | . | . | . | . | . | . | . | . | 18 |
| Belly | 21. iii. 36 | White | 327 | 80 | 6 | 126 | 184 | 11 | . | . | . | . | . | . | . | . | 16 |
| | | Brown | 43 | 10 | 3 | 17 | 23 | . | . | . | . | . | . | . | . | . | 15 |
| | | Black | 40 | 10 | . | 2 | 18 | 18 | 3 | . | . | . | . | . | . | . | 21 |
| T [illegible] | 25. i. 36 | White | 307 | 70 | . | 2 | 61 | 93 | 79 | 56 | 11 | 5 | . | . | . | . | 26 |
| | | Brown | 74 | 17 | . | 7 | 34 | 25 | 7 | 1 | . | . | . | . | . | . | 21 |
| | | Black | 57 | 13 | . | 2 | 5 | 18 | 17 | 13 | 1 | 1 | . | . | . | . | 27 |

(1) Includes light brown, brown and dark brown. (2) Includes black and deep black.

Table 11. *Lamb No. 55. Distribution in diameter* ($7{\cdot}5 = 10\mu$) *of different coloured fibres in the fleece in different body regions and at different ages.*

| Body region | Date | Colour of fibre | Fibres Total | Fibres % | No. of fibres with diameter of −8 | −13 | −18 | −23 | −28 | −33 | −38 | 43 | 48 | 53 | 58 | 63 | 68 | 73 | Av. fibre diameter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shoulder | 25. i. 36 | White | 286 | 57 | 1 | 88 | 163 | 31 | 3 | . | . | . | . | . | . | . | . | . | 17 |
| | | Brown (1) | 108 | 21 | 2 | 85 | 18 | 2 | 1 | . | . | . | . | . | . | . | . | . | 14 |
| | | Black (2) | 9 | 2 | . | 2 | 5 | 1 | . | . | 1 | . | . | . | . | . | . | . | 20 |
| | 21. iii. 36 | White | 371 | 93 | . | 43 | 195 | 117 | 15 | 1 | . | . | . | . | . | . | . | . | 19 |
| | | Brown | 30 | 7 | 3 | 19 | 8 | . | . | . | . | . | . | . | . | . | . | . | 14 |
| | | Black | 0 | 0 | . | . | . | . | . | . | . | . | . | . | . | . | . | . | — |
| | 16. v. 36 | White | 408 | 97 | 2 | 57 | 203 | 145 | 21 | 1 | . | . | . | . | . | . | . | . | 20 |
| | | Brown | 11 | 3 | . | 10 | 1 | . | . | . | . | . | . | . | . | . | . | . | 13 |
| | | Black | 0 | 0 | . | . | 1 | . | . | . | . | . | . | . | . | . | . | . | 18 |
| Leg | 25. i. 36 | White | 208 | 52 | . | 19 | 105 | 69 | 13 | 2 | . | . | . | . | . | . | . | . | 20 |
| | | Brown | 127 | 32 | 3 | 45 | 68 | 9 | 1 | 1 | . | . | . | . | . | . | . | . | 17 |
| | | Black | 64 | 16 | . | 12 | 19 | 6 | 9 | 3 | 4 | 4 | 5 | 1 | . | 1 | . | . | 26 |
| | 21. iii. 36 | White | 343 | 83 | . | 4 | 63 | 127 | 101 | 31 | 15 | 1 | 1 | . | . | . | . | . | 25 |
| | | Brown | 35 | 9 | . | 12 | 13 | 6 | . | . | . | 2 | 2 | . | . | . | . | . | 20 |
| | | Black | 35 | 8 | . | 1 | 2 | 8 | 9 | 6 | 3 | 3 | . | 3 | . | . | . | . | 31 |
| | 16. v. 36 | White | 392 | 93 | 1 | 10 | 85 | 155 | 113 | 25 | 3 | . | . | . | . | . | . | . | 24 |
| | | Brown | 3 | 1 | 1 | 2 | . | . | . | . | . | . | . | . | . | . | . | . | 11 |
| | | Black | 27 | 0 | . | 4 | 8 | 12 | 2 | . | . | 1 | . | . | . | . | . | . | 21 |
| Belly | 21. iii. 36 | White | 286 | 72 | 1 | 51 | 171 | 37 | 8 | 5 | 6 | 6 | 1 | . | . | . | . | . | 19 |
| | | Brown | 42 | 11 | 1 | 23 | 10 | 4 | 1 | . | 3 | . | . | . | . | . | . | . | 17 |
| | | Black | 70 | 17 | 1 | 5 | 27 | 18 | 10 | 6 | 3 | . | . | . | . | . | . | . | 23 |
| Tail | 25. i. 36 | White | 155 | 39 | 5 | 1 | 2 | 24 | 28 | 26 | 19 | 29 | 11 | 6 | 4 | . | . | . | 34 |
| | | Brown | 95 | 24 | . | . | 17 | 24 | 4 | 5 | 10 | 14 | 11 | 6 | 2 | . | 1 | 1 | 34 |
| | | Black | 146 | 37 | . | 3 | 6 | 16 | 28 | 20 | 21 | 13 | 9 | 11 | 7 | 1 | 1 | 1 | 36 |

(1) Includes light brown, brown and dark brown.
(2) Includes black and deep black.

least to some extent, those which might control the rapidity of the division of the cells.

On the other hand, the follicles capable of producing different shades in the fibres may undergo changes by factors acting upon the follicle, not during its formation but at a more or less advanced stage of development of the lamb, and are more strongly influenced by physiological factors than the other follicles. We shall term the factors acting upon the follicles with white fibres "qualitative" factors and those acting upon follicles capable of producing different shades, "quantitative" factors. There are also the fibres that are clearly black at the tip and then quite white starting from a well-defined point. As regards these the interpretation of the phenomenon is more complex, though it is probable that it, like the others, is connected with the rapidity of development.

Tables I and II give the reader an idea of how the various shades and colours are distributed in the regions of the skin and according to the fineness of the fibres at different stages of development. Passing from the regions with the earlier development to those with a later development it is seen that for each stage there is, at a given time in the development, a corresponding definite shade of fibres which are more numerous than the others but which, above all, are seen to be more regularly distributed so that they tend to give a regular frequency curve with one peak. Furthermore, these shades "characteristic" of each region become in general darker and darker as one passes from the "early" to the "late" regions.

The shades that show the most regular curves for the various regions are as follows:

| Lamb | Region | Date | Colour which displays the most regular curve |
|---|---|---|---|
| Lamb No. 54 | Shoulder | 25. i. 36 | Light brown and brown |
| | Leg | 25. i. 36 | Brown |
| | Belly | 21. iii. 36 | Dark brown |
| | Tail | 25. i. 36 | Deep black |
| Lamb No. 55 | Shoulder | 25. i. 36 | Brown |
| | Shoulder | 21. iii. 36 | Brown and dark brown |
| | Leg | 25. i. 36 | — |
| | Leg | 21. iii. 36 | Brown and dark brown |
| | Belly | 21. iii. 36 | Brown and black |
| | Tail | 25. i. 36 | Black |

In our opinion this shows once again the relation that exists between the development of the region and the capacity for the production of colour in the fibres. The white fibres always exhibit rather regular curves.

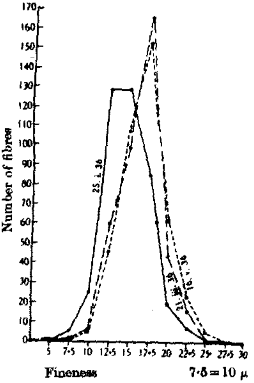
The percentage of fibres of the different shades varies greatly from one grade of fineness to another. Hence the factors acting upon the shades

of the colours do not behave in the same way as regards the fibres of different degrees of fineness. The different trend of the curves representing the fibres of black colour (see Tables I and II) and those of lighter colouration suggests that the nature of the factor (or factors) determining the colour deep black must be different from that of the lighter shades. This finding is specially marked in the tail region of lamb No. 55. It seems to us probable that the pigment of the deep black fibres is not only quantitatively but also qualitatively different from that of fibres with lighter colours. Thus the brown fibres probably become lighter simply owing to the dilution of the pigment; whereas in the deep black fibres there is possibly a physico-chemical or a chemical change in the pigment or the substance forming its substratum.

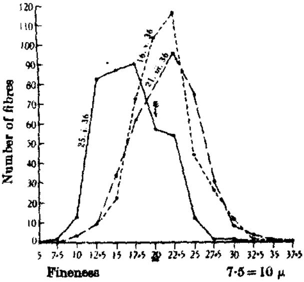
### (*b*) *Medullated fibres*

We have divided the fibres according to the greater or smaller proportion of medulla observed in them on microscopic examination into four types: (1) fibres exhibiting no medulla (type A); (2) fibres with an intermittent medulla (type B); (3) fibres with a continuous medulla occupying about 80 % of the thickness of the fibre (type C); and (4) fibres having over 80 % medulla (type D).

The reader may obtain an idea of the proportion of these various types




Text-fig. 1 (*a*). Lamb 54, shoulder.




Text-fig. 1 (*b*). Lamb 54, leg.

in the different regions of the skin by studying Table III, noting, however, that the figures are only approximate owing to the difficulty of distinguishing the medulla in fibres of shades that are too dark.

It is interesting to observe that in the fibres possessing a medulla the fibres tend to increase in diameter by an increase in the diameter of the medulla and not by increasing the size of the cortical portion which tends to remain practically the same.

It should also be noted that in the belly region there are relatively few fibres with a medulla although it is a region that develops late.

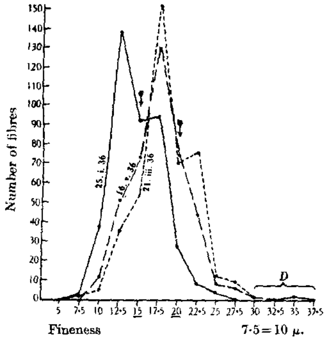
Table III. *Distribution in diameter* ($7{\cdot}5 = 10\mu$) *of the medullated and non-medullated fibres of different sorts in the different regions of the body at different ages*

| Lamb | Body region | Date | Type of fibre | No. of fibres with diameter of −8 | −13 | −18 | −23 | −28 | −33 | −38 | −43 | −48 | −53 | −58 | −63 | −68 | −73 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 54 | Shoulder | 25. i. 36 | A | 5 | 152 | 212 | 26 | 1 | . | 1 | . | . | . | . | . | . | . |
| | | | C | . | 1 | . | . | . | . | . | . | . | . | . | . | . | . |
| | Shoulder | 21. iii. 36 | A | 3 | 66 | 263 | 69 | 3 | . | . | 1 | . | . | . | . | . | . |
| | Shoulder | 16. v. 36 | A | 1 | 52 | 258 | 85 | 5 | . | . | . | . | . | . | . | . | . |
| | Leg | 25. i. 36 | A | 2 | 96 | 161 | 64 | 3 | . | . | . | . | . | . | . | . | . |
| | | | B | . | . | 11 | 34 | 3 | . | . | . | . | . | . | . | . | . |
| | | | C | . | . | 5 | 15 | 7 | 1 | . | . | . | . | . | . | . | . |
| | Leg | 21. iii. 36 | A | 2 | 12 | 94 | 157 | 86 | 9 | 1 | . | . | . | . | . | . | . |
| | | | B | . | . | 1 | 17 | 18 | 2 | . | . | . | . | . | . | . | . |
| | | | C | . | . | . | . | 1 | . | . | . | . | . | . | . | . | . |
| | Leg | 16. v. 36 | A | . | 11 | 94 | 219 | 70 | 12 | . | . | . | . | . | . | . | . |
| | Belly | 21. iii. 36 | A | 8 | 146 | 239 | 27 | 3 | . | . | . | . | . | . | . | . | . |
| | Tail | 25. i. 36 | A | . | 9 | 100 | 136 | 74 | 31 | 2 | 4 | . | . | . | . | . | . |
| | | | B | . | . | . | . | 20 | 26 | 4 | 1 | . | . | . | . | . | . |
| | | | C | . | . | . | . | 9 | 13 | 5 | 1 | . | . | . | . | . | . |
| 55 | Shoulder | 25. i. 36 | A | 3 | 175 | 182 | 13 | 4 | . | 1 | . | . | . | . | . | . | . |
| | | | B | . | . | 4 | 1 | . | . | . | . | . | . | . | . | . | . |
| | Shoulder | 21. iii. 36 | A | 3 | 62 | 203 | 120 | 15 | 1 | . | . | . | . | . | . | . | . |
| | | | B | . | . | 1 | . | . | . | . | . | . | . | . | . | . | . |
| | Shoulder | 16. v. 36 | A | 2 | 40 | 205 | 145 | 21 | 4 | 4 | 4 | 5 | 1 | 1 | . | . | . |
| | | | C | . | . | . | . | . | 2 | . | . | . | . | . | . | . | . |
| | Leg | 25. i. 36 | A | 2 | 78 | 182 | 46 | 14 | . | . | . | . | . | . | . | . | . |
| | | | B | 1 | 2 | 10 | 25 | 5 | . | . | . | . | . | . | . | . | . |
| | | | C | . | . | 1 | 9 | 4 | . | . | . | . | . | . | . | . | . |
| | Leg | 21. iii. 36 | A | . | 17 | 74 | 102 | 59 | 15 | 9 | 2 | . | 2 | 1 | . | . | . |
| | | | B | . | . | 4 | 36 | 34 | 9 | 2 | . | . | . | . | . | . | . |
| | | | C | . | . | . | 3 | 17 | 11 | 5 | 2 | 1 | . | . | . | . | . |
| | | | D | . | . | . | . | . | 1 | 3 | 2 | . | 1 | . | . | . | . |
| | Leg | 16. v. 36 | A | 2 | 16 | 93 | 166 | 109 | 24 | 3 | 1 | . | . | . | . | . | . |
| | | | B | . | . | . | 1 | 5 | 1 | . | . | . | . | . | . | . | . |
| | Belly | 21. iii. 36 | A | 3 | 79 | 208 | 58 | 19 | 8 | 4 | . | . | . | . | . | . | . |
| | | | C | . | . | . | . | . | 3 | 8 | 6 | 1 | . | . | . | . | . |
| | Tail | 25. i. 36 | A | 4 | 4 | 25 | 63 | 52 | 40 | 31 | 21 | 8 | 14 | 9 | 1 | 2 | 2 |
| | | | B | 1 | . | . | 1 | 5 | 5 | 1 | 2 | 3 | 1 | . | . | . | . |
| | | | C | . | . | . | . | 2 | 14 | 19 | 31 | 20 | 7 | 4 | . | . | . |
| | | | D | . | . | . | . | . | . | . | 1 | . | . | . | . | . | . |

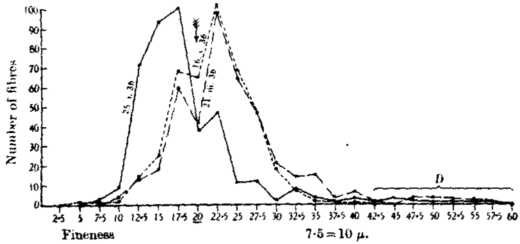
A = Fibres with no medulla.
B = Fibres with an intermittent medulla.
C = Fibres with a continuous medulla occupying under 80 % of the fibr thickness.
D = Fibres having a medulla of over 80 % of their thickness.

### (c) *Fineness*

It is seen in Text-fig. 1 *a* and 1 *b* showing the frequency curves for the degrees of fineness for the shoulder and the leg, respectively, of lamb No. 54 and in Text-fig. 2*a* and 2*b* representing the corresponding data




Text-fig. 2 (*a*). **Lamb 55, shoulder.**




Text-fig. 2 (*b*). **Lamb 55, leg.**

for lamb No. 55 that there is a wide difference between the degrees of fineness of the fibres at 1 month old and those which grow during the later periods of development. In Text-fig. 1 *a* we see that the frequency curve for the shoulder on the dates 21 March 1936 and 16 May 1936 remains almost the same; hence this region already no longer varies at

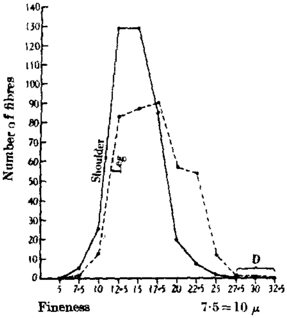
this period for lamb No. 54. The curve for lamb No. 55 at the shoulder on the same dates, Text-fig. 2*a*, also appears to show little development, if we consider the general trend of the curve without considering the details. Note, however, that (*a*) the two curves, those representing the wool sheared on 21 March 1936 and that on 16 May 1936, exhibit a greater difference *inter se* than those of lamb No. 54 in the region with the coarse fibres (indicated by a *D* in the figure); (*b*) the curve for 21 March 1936 (and similarly that for 25 January 1936) exhibits a depression at the place marked with an arrow, whilst the curve for 16 May 1936, shows no depression and resembles the curves for lamb No. 54 for the same dates. Judging from this, therefore, development as regards fineness in this animal for the shoulder would not be completed till between the third and fifth months after birth, whereas in the other animal development should already be almost completed at this period. It is an interesting fact that the depressions of importance in all the curves are always found opposite the number 20 and sometimes 15 representing the scale of fineness. The same phenomenon is found in adult animals showing one less cross of Suffolk blood.

The same methods applied to the wool of the leg, using the same criterion to estimate the "earliness" of a region, show us that the leg of lamb No. 54, Text-fig. 1*b*, and the leg of lamb No. 55, Text-fig. 2*b*, also exhibit the same differences, but more accentuated. Thus the curve representing the leg of No. 54 exhibits a depression on 25 January 1936 and those for the dates 21 March 1936 and 16 May 1936 no longer show a depression. They display a similar trend and show little difference in the region of the coarse fibres (*D*); whereas lamb No. 55, Text-fig. 2*b*, shows similar trends on the dates 21 March 1936 and 16 May 1936 and the curves display, like the curve for the wool at birth, a well-marked depression, thus showing that the fibres have not entirely completed their development as regards the degrees of fineness. On the other hand, the difference (*D*) between the degrees of fineness in the regions of the curve with coarse fibres is very great.

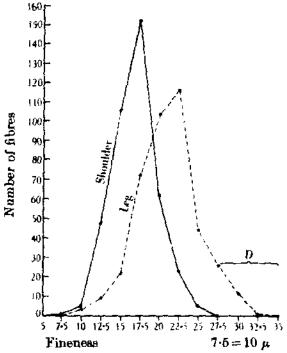
These facts lead us to the view that when one wishes to determine the value of the fleece of sheep for breeding, on the basis of the frequency curves for fineness, one must eliminate the individuals displaying depressions in the curves, and deviations in the regions of the coarse fibres. In this respect a study by means of frequency curves is one of more value than a study by means of calculations of the average fineness, coefficient of variation, etc.

### (*d*) *Uniformity of fibre size*

From the commercial standpoint the importance of obtaining a uniform fleece is well known. Breeders obtain an idea of the uniformity of the fleece by examination of a part characterized by "early" development (shoulder) and a part characterized by late development (the leg). Although in principle the method is good, since, without knowing it, they compare "early" regions with "late" ones, the method is subject to a certain criticism in the light of the facts considered above. Thus in a breed in which the leg is highly evolved it would be best, for example, to examine a part still "later" such as the tail. We observe in Text-figs. 3*a*



Text-fig. 3 (*a*). Lamb 54, 25. January 36.

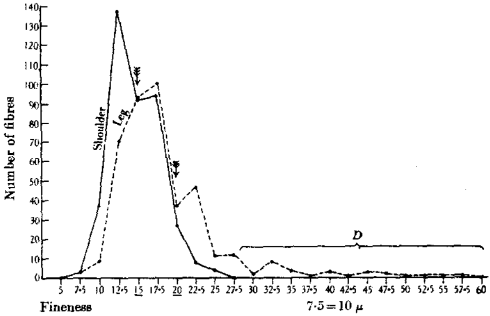


Text-fig. 3 (*b*). Lamb 54, 16 May 36.

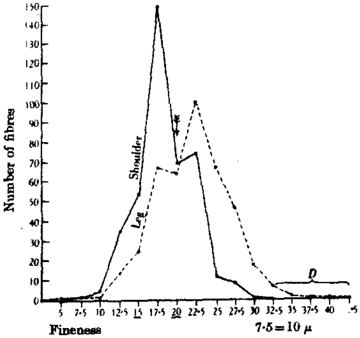
and 3*b* representing the frequency curves for the shoulder and the leg of lamb No. 54 and Text-figs. 4*a* and 4*b* representing those for lamb No. 55 that the differences between the regions are not the same at both periods—a fact which was to be expected since the "early" regions develop more rapidly. The curves for lamb No. 54 (good lamb) for the date 16 May 1936 display, when reduced to numerical averages, differences as great and even greater between the shoulder and the leg than for lamb No. 55 (inferior lamb), and this is so as regards the fineness and the uniformity. On the other hand, if one considers only the deviations for the regions of the curves with coarse fibres (*D*), it is seen that in the curves of lamb No. 54 this difference is small and for No. 55 very large.

### III. The best region for judging the value of the wool in lambs

The differences between the qualities of the wool from the same region of the skin in two different individuals is not the same (or, in other words, does not remain constant) during the course of development. This fact can be seen by comparing Text-figs. 1 *b* and 2 *b*.




Text-fig. 4 (*a*). Lamb 55, 25 January 36.




Text-fig. 4 (*b*). Lamb 55, 16 May 36.

When one is judging the wool value of a future breeding animal on the basis of the examination of its fleece as a lamb the best part for such an examination will depend upon: (1) the stage of development—more or less advanced—at the time of examination; (2) the "earliness" of the breed examined; (3) the degree of improvement attained by the breed as regards wool qualities. At an early stage of development the two regions are very similar, later one of these regions developing more rapidly in the early maturing animal than in the other, they will display the maximum of differences, one of these regions still retaining the coarse fibres and the other (those of the improved animal) having shed them. Later still the regions resemble each other a little, but then the differences that remain are final.

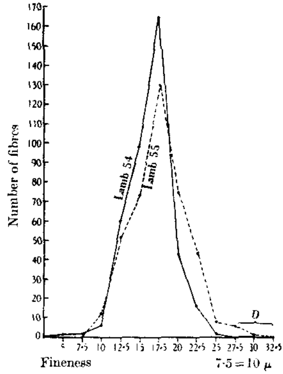
It is of considerable interest to breeders to determine which is the best region for the examination of lambs at the time when the tails are being docked. The best animal will be the one which develops more rapidly in the "latest" developing of these regions. It should be noted that examination of the lamb as compared with the examination of the adult has the advantage of giving us some idea of its genetic constitution, revealing to us defects which would be concealed later; one might therefore term this method a "phaeno-genetic" method much less complicated, certainly, than the methods used or suggested up to the present.

In this case the lambs from the cross (Suffolk × Suffolk (Border Leicester-Cheviot)) display at the time of docking (one month old) the maximum of differences in the tail region, as the reader can observe from an examination of Text-fig. 5*b* and a comparison of it with Text-fig. 5*a* representing the differences for the shoulder. Pl. VI shows us a classification made with a series of tails of this cross classed according to the wool value of the lambs. One can very clearly see the difference merely by macroscopic examination. For a better estimation of the value it is well to note that the middle of the tail is the site of the better wool. The edges are very hairy and the end still more so. If the tail is turned round with the underside outwards the hairs are still more numerous and coarser.

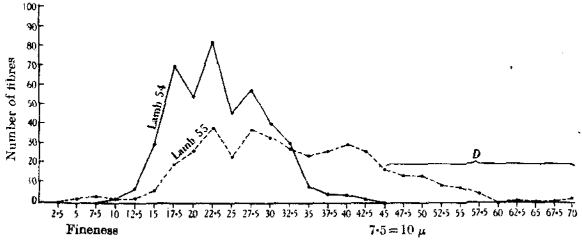
## Summary

1. A study has been made of the relative development of some regions in the fleece of twin lambs of a Suffolk ♂ × Suffolk (Border Leicester-Cheviot) ♀ cross during growth. The different regions develop in the following order: shoulder → leg → belly → tail. It is concluded that an

improved animal is one in which the latest developing part of the fleece has the least possible number of undesirable fibres at the earliest possible




Text-fig. 5 (*a*). Shoulder.




Text-fig. 5 (*b*). Tail.

age. The breeds of sheep begin their wool improvement by changes in the earliest developing regions (shoulder) and finish it in the latest (tail).

2. The properties of the fleece studied were the colour of the fibres, the medullated fibres, the fineness of the fibres, and uniformity of fibre size. In general a relationship is found between the stage and rapidity of development of the area, and the properties of the fibre. The intensity

of the colour is stronger during the early active stages of development. There is a tendency to lose some kinds of coloured fibres, and to diminish the intensity of the colour in others as the development of each region in which the fibres grow proceeds.

The medullated fibres appeared in greatest quantities in the later developing regions, except the belly which presents few medullated fibres.

The staple is much finer at the first month of growth than in the later stages. Frequency curves of fibre size are of more value than average fibre size in estimating the value of the fleece.

3. It is suggested that the examination of the fleece of the lamb will give some idea of its genetic constitution and will reveal defects which would be concealed in the adult. Such a "phaeno-genetic" selection can be made best in a "late developing" area of the body such as the tail of the lamb at one month old.

I wish to express my gratitude to Dr Hammond who suggested this problem to me, indicated the techniques to use, and placed the experimental material at my disposal, and to whose kindness and hospitality I am indebted for having enabled me to carry out the work in his laboratory.

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(*Received* 9 *December* 1937)

Fig. 1 (*a*). 2 weeks old.

Fig. 1 (*b*). 2 months old.

Fig. 1 (*c*). 3 months old.

Fig. 1 (*d*). 3 months old.

Lamb No. 55.

Fig. 2 (*a*). 2 weeks old.

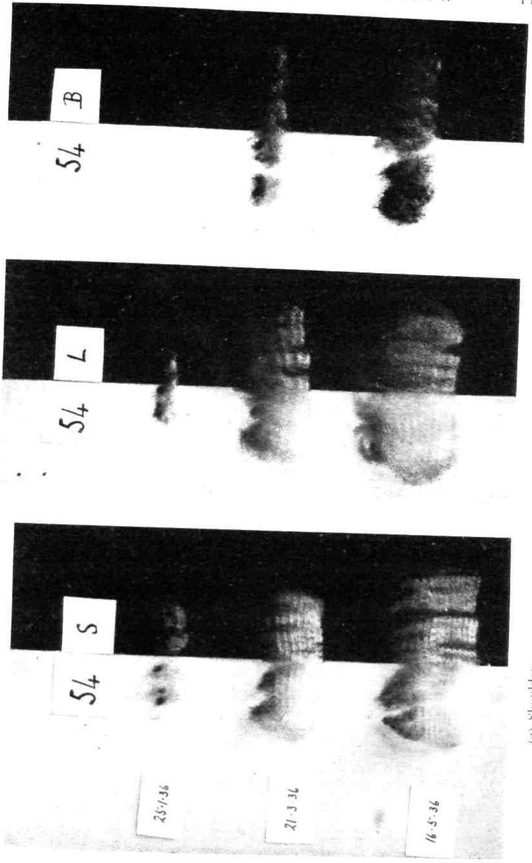
Fig. 2 (*b*). 2 months old.

Fig. 2 (*c*). 3 months old.

Fig. 2 (*d*). 3 months old.

Lamb No. 54.

Plate IV




(a) Shoulder.

(b) Leg.

(c) Belly.

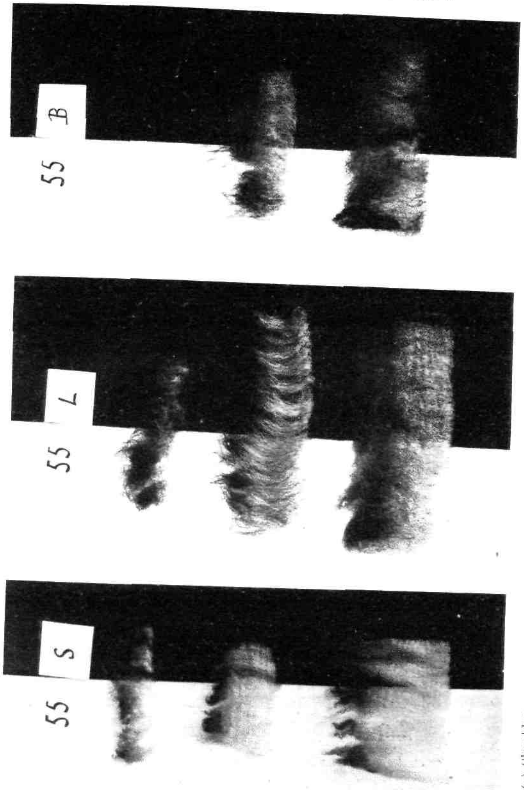
Lamb No. 54.

1 month

3 months

5 months

Plate V




1 month

3 months

5 months

(a) Shoulder.

(b) Leg.

(c) Belly.

Lamb No. 55.

Lamb No. 52 53 54 55

Lambs' tails at 1 month old.

# A STUDY OF THE EFFECT OF FEEDING OILS TO DAIRY COWS AND OF THE VALUE OF THE LATIN SQUARE LAY-OUT IN ANIMAL EXPERIMENTATION

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(With Two Text-figures)

Many investigators have attempted to determine the effect of various foods on the yield and composition of cows' milk. The general literature on the subject is so vast and so well known that it is not proposed to discuss it in the present paper. The main object of much of the work has been to find foods which would increase the percentage and yield of butterfat. In Germany, Hansen (1928), after an extensive series of experiments, concluded that of the common foods, palm kernel cake, coconut cake and maize-germ meal increased butterfat percentage without decreasing yield of milk. The subject has been followed up more recently by Allen (1934) and Sheehy (1933) who have studied the effect of adding oils to dairy rations. Allen, in a series of short-term experiments, found that butterfat, lard, tallow, linseed oil, cotton-seed oil, corn oil, peanut oil, soya-bean oil and coconut oil all increased the percentage and yield of butterfat. He found that the effects were produced between 12 and 24 hr. after the commencement of fat or oil feeding, and that the increase of butterfat yield was equivalent to 10–20 % of the increase in butterfat intake. These results were not in agreement with those obtained by Sheehy, who found that olive oil, linseed oil, cotton-seed oil, coconut oil, ground-nut oil, palm-nut oil, soya-bean oil, beef fat and whale oil had no effect on the yield of butterfat; he found that cod-liver oil (fed at the rate of 6 oz. per cow daily) had a depressing effect which lasted for several days after cod-liver oil was withheld. Both Allen and Sheehy found considerable variation among their cows in the reaction to the oils fed. More recently McCandlish & Struthers (1935), working with only eleven cows in all, failed to find any increase in yield of milk or fat, when butterfat was added to the rations. There is, therefore, considerable contradiction in the literature on the subject, and it was felt that further work should be done; consequently, between March 1935 and March 1937, seventeen separate experiments were completed, using the cows of the herd on the Cambridge University Farm.

## Experimental method

As stated above, other workers' results indicate that, where any effect of adding oil to the rations occurred, it was produced very rapidly, and it was therefore decided to adopt the Latin square lay-out, despite the fact that it necessitated frequent and rapid changes over. The principle involved in the Latin square lay-out was that there were "$n$" cows, "$n$" periods and "$n$" levels of fat feeding (including the control). In all cases except one the value of "$n$" was 4, and except for two further experiments the unit period was 5 days. Thus, fourteen of the experiments were exactly alike in lay-out, an example of which is given in Table I. It will be seen that in the first week of that experiment Russett 7th received an addition of 1½ lb. of oil to her ration, Star 5th 1 lb., Maud 10th ½ lb., whilst Daisy 5th received no oil during the week. It will be appreciated that the lay-out ensures that each cow shall provide her own control, and this is regarded as of fundamental importance in view of the fact that a very high proportion of the variation in such experiments is ascribable to variations between individual cows. The first feed to which oil was added was always the last feed on Sunday, and the treatment continued till the penultimate feed on the following Friday: this left 2 days, during which

Table I. *Example of a Latin square lay-out*

| | Cows | | | |
|---|---|---|---|---|
| Weeks commencing | Russett 7th | Maud 10th | Daisy 5th | Star 5th |
| 25. iii. 35 | 1½ | ½ | 0 | 1 |
| 1. iv. 35 | 0 | 1½ | 1 | ½ |
| 8. iv. 35 | ½ | 1 | 1½ | 0 |
| 15. iv. 35 | 1 | 0 | ½ | 1½ |

*Figures in the body of the table denote lb. of oil added daily to the cows' rations.*

no cow received any oil, before the treatments of the following week commenced. The unit period over which records were taken was Monday morning till Friday night inclusive. Most of the experiments were worked out on two unit periods—the one just given and also on the last 3 days (Wednesday to Friday), inclusive, of the period: it was felt that a comparison of the two would show whether there was any appreciable delay in the development of the effect. It can be said at once that, over the whole series, results based on 5 and on 3 days agreed very closely, and in the tables given below the full period has been taken as the unit: it will be realized that in so far as there is any delay in action of the oil the

tendency has been to reduce the significance. Figs. 1 and 2 suggest that there was some delay. The results illustrated in Fig. 1 were from all five experiments in which positive results were obtained in regard to yield of butterfat. When the three levels of fat feeding were averaged, the increase in yield on the first day after initiation of feeding was only 6 % compared with 10–14 % for the remaining days. Thus, though there is some evidence that the full effect was not developed in the first day, the slight reduction in response on that day, together with the agreement of results calculated

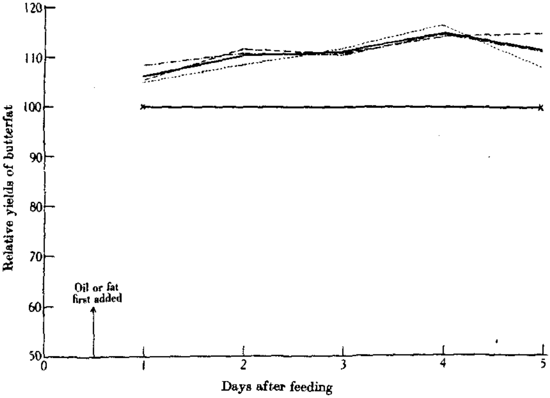


Fig. 1. Average results of five short-term experiments showing significant increases in butterfat yield when fat or oil was added to the ration. 1 cotton-seed oil; 2 butter; 1 lard; 1 palm oil.

x——— Control    -------- Single dose of oil
------- Double dose    -·-·-·- Treble dose
——— Mean of single, double and treble doses

on 5 days and on the last 3 days, have been held to justify the presentation of results only for the whole period. In Fig. 2 the combined results for two experiments with cod-liver oil, which gave definite negative results, are illustrated, and it will be seen that the response on the first day was very different from that on the remaining days of the period. In both experiments the yield of fat was increased (in one case significantly) on the first day after the beginning of the feeding of cod-liver oil, but thereafter there was a marked and apparently progressive fall

throughout the remainder of the period. For the sake of uniformity the results will again be presented for the full 5 days, though this will clearly understate the fully developed fall in yields; it will be seen, however, that even with this inclusion the results are highly significant.

In twelve of the fourteen short-term experiments the milk of each cow was sampled and tested for fat at every milking, and results are available for milk yield, butterfat percentage and butterfat yield for each milking separately. In the other two experiments the method of sampling

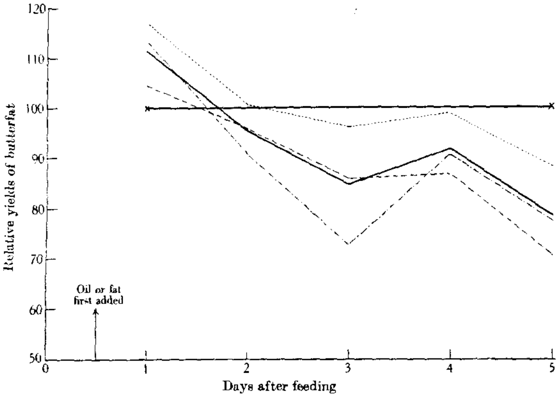


*Fig. 2. Average results of two short-term experiments with cod liver oil showing* significant decreases in butterfat yield when oil was added to the ration.

x——— Control; - - - - - Single dose of oil; - - - - - Double dose; - - - - - Treble dose; ——— Mean of single, double and treble doses

was changed. At each milking the milk from each cow was placed separately in a sampling bucket carrying a glass tube at the side, from which it was possible to obtain an aliquot sample: preliminary tests showed that the sample obtained by this method was not only accurately proportioned to the amount of milk, but also closely representative of its composition. The morning, afternoon and, in the case of thrice-milked cows, noon samples of each cow were separately accumulated over the unit period, preservative being added: this reduced the amount of laboratory testing

very considerably, and it was possible in these two experiments to obtain not only the fat percentage of the samples but also the solids-not-fat percentages.

The unit period in the above fourteen experiments was only 5 days, and might be regarded as being unduly short to permit of the full development of the effects. In two further experiments the unit period was doubled, as also was the length of the change-over periods. In one case the experiment was again a $4 \times 4$ Latin square and consequently occupied a period of 2 months, whilst in the other an extra level of fat feeding was introduced, making a $5 \times 5$ Latin square which occupied 10 weeks. In both cases the sampling bucket was used, but samples were accumulated for only half the unit period; samples obtained in the first 5 days of the unit period have been designated "A" samples, and those from the second 5 days "B" samples, and all results were calculated separately for A and B periods, and for the total of both periods. The object of this was to determine the rapidity and the persistency of the effects, but there was in general little difference between results from A and B samples, and consequently only those from total unit periods need be considered.

Finally, one long-period experiment was conducted in which unit periods and periods of change were again doubled: the lay-out was again a $4 \times 4$ Latin square, and occupied 16 weeks. Fortunately all four cows milked consistently throughout this long period. In this case the unit feeding period was divided into three, A and B samples covering 7 days each, and C samples 6 days. Again, the results from the separate samples agreed, and in consequence only the results are given for the whole periods.

In the medium, the long and some of the short-term experiments the cows were weighed at each change-over, but, with the inevitable inaccuracy in the weighing, any real variation over the short periods concerned was too small to be detected.

The modern technique of field experimentation is designed to give valid and low errors, and it is often desirable to compare the precision of work with various crops and in various conditions. For this purpose a useful figure is provided by expressing the standard error of one plot (that is, the root mean square from the error line of the analysis of variance) as a percentage of the general mean. In carefully conducted experiments with crops, plot yields usually give figures of 10 or under, most cases lying between 4 and 14. It is of interest to see what the corresponding figures were in the case of these experiments, and Table II gives the distribution of the "error per cent" figures for milk yield, butterfat

per cent and butterfat yields for the short-term experiments here described. In addition to giving the figures for all the milkings the table gives those when the experiments were worked out separately on morning and evening, and, in the six cases where the cows were milked three times daily, the noon milkings. The errors shown in the table may be claimed to be satisfactory, as although they vary from 1 to 16, the mean value is approximately 6, which compares favourably with experiments on crops: this figure of 6, it should be noted, is for single cows over a period of only 5 days. As would be expected, the errors were slightly lower when all milkings were combined than for separate milkings, though the difference was not great; there was no apparent difference either in value, or in dispersion, of figures between morning, noon and evening milkings. With the medium and long-term experiments the figures were of the same order as in Table II as regards butterfat percentage, but were somewhat higher (averaging about 9) in the case of milk yield. The percentage of solids-not-fat in the milk was very constant, the "error per cent" figures for this variable averaging only 1·4; with solids-not-fat yield the errors were of the same order as shown in Table II.

Table II. *Distribution of percentage errors in fourteen short-term experiments*

| Error as % of G.M. | Milk yield: Separate milkings | Milk yield: Total of all milkings | Butterfat percentage: Separate milkings | Butterfat percentage: Total of all milkings | Butterfat yield: Separate milkings | Butterfat yield: Total of all milkings |
|---|---|---|---|---|---|---|
| 0– | — | — | — | — | — | — |
| 1– | 1 | 2 | — | — | — | — |
| 2– | 8 | 4 | 3 | — | — | 1 |
| 3– | 5 | 1 | 1 | 1 | 1 | 2 |
| 4– | 2 | 2 | 2 | 5 | 2 | 5 |
| 5– | 6 | 2 | 5 | 2 | 7 | 1 |
| 6– | 4 | 1 | 7 | 1 | 3 | — |
| 7– | 4 | 1 | 3 | 4 | 2 | — |
| 8– | 1 | — | 4 | — | 4 | 3 |
| 9– | — | 1 | 1 | — | 5 | — |
| 10– | 1 | — | 3 | 1 | 2 | — |
| 11– | 2 | — | 3 | — | 3 | 2 |
| 12– | — | — | 1 | — | 1 | — |
| 13– | — | — | — | — | 2 | — |
| 14– | — | — | — | — | 1 | — |
| 15– | — | — | 1 | — | 1 | — |
| *Total* | 34 | 14 | 34 | 14 | 34 | 14 |

Although the above errors may be regarded as satisfactory, it is felt that it would be an improvement to have more than one cow as a unit. If two or three cows could be provided for each cell of the Latin square it is very probable that many chance fluctuations would be smoothed out,

with consequent further lowering of errors. $4 \times 4$ Latin squares suffer from the fact that they only provide 6 degrees of freedom for error; probably the most efficient procedure would be to use larger squares with, say, two cows as a unit. From the experience in these experiments it appears that there is little point in keeping milkings separate and, if in addition the bulk sampling procedure described above is used, little chemical and statistical labour is involved. In the present series of experiments a unit period of 5 days gave similar results to longer periods, and though slow developing effects may demand longer periods, lengthening them must necessarily increase the chances of illness and mishaps with the cows; in two cases among the experiments here described, figures were lacking for one cell of the Latin square (one case through illness, and the other through premature drying off of a cow) and had to be calculated by the "missing plot" method. It is of course fully appreciated that the Latin square lay-out must be limited in its use to those experiments where effects are rapidly produced and wear off very quickly when the treatment is discontinued. If effects are slow, possibly due to an animal not taking to a new food for a few days, or if the effect of the treatment lingers on, there must be much longer periods allowed for changes over, and this will seriously impair the efficiency of the lay-out, by spreading out the observational periods, and thus reducing the advantage gained by using each cow as her own control.

In all these experiments the treatments were so chosen that the increase in fat fed was constant over the whole range: for example, in the cases illustrated in Fig. 1 the four treatments were 0, ½, 1 and 1½ lb. of oil per cow per day, and in the case of cod-liver oil they were 0, 3, 6 and 9 oz. daily. In analysing the degrees of freedom for treatment in such an experiment, it is clearly possible to take 1 degree of freedom for the regression (of yield on amount of fat fed) and the other two for the deviations from the regression or, alternatively, to take one degree of freedom for the comparison of Fat *v.* 0, leaving the other two for variations among the amounts of fat fed. In the event of there being a fairly constant rise in yield with increasing amounts of fat fed, the former method will give a higher degree of significance, whereas if the single dose of fat is as effective as the double and the treble, then the latter method produces the greater degree of significance. It is clear that either method is perfectly justifiable, and it has been considered permissible to choose the one which most clearly expressed the result.

The cows used were selected from the ordinary members of the Cambridge University Farm herd of Dairy Shorthorns. In general the

principle was to group into Latin squares cows which were as alike as possible in stage of lactation, etc., but experience showed that the method of control was so good that quite wide differences could be allowed, without any appreciable increase in experimental error; naturally it is imperative to avoid cows which may by any chance commence to "dry off" before the conclusion of the experiment. The practice with the herd is to milk cows yielding more than 3 or 4 gal. three times daily, and in all the experiments, except one, all the cows in a particular experiment were milked a constant number of times daily throughout the period. The exception was the one 5 × 5 Latin square, which included two cows milked three times daily, and three cows milked twice daily, throughout the experiment. Although it was the aim in building up a group of cows to select those of roughly the same level of production at the time, there were, in some cases, quite large discrepancies between the yields of individual members of the group; the same was true with regard to the

Table III. *Summarized description of the experiments*

| *Code* no. | *Fat or oil* used | *Date* started | Treatments—Amounts fat or oil added (lb.) Control | Single | Double | Treble | No. of milkings *per* day | Unit *period* (days) |
|---|---|---|---|---|---|---|---|---|
| I | Cotton-seed oil | 18. iii. 35 | 0 | ½ | 1 | 1½ | 2 | 5 |
| II | Cotton-seed oil | 25. iii. 35 | 0 | ½ | 1 | 1½ | 3 | 5 |
| *III* | *Butter* | *29. iv. 35* | 0 | ¾ | 1½ | 2¼ | 3 | 5 |
| IV | Butter | 13. x. 35 | 0 | ¾ | 1½ | 2¼ | 3 | 5 |
| V | Butter | 13. i. 36 | 0 | ¾ | 1½ | 2¼ | 2 | 5 |
| VI | Lard | 21. x. 35 | 0 | ¾ | 1½ | 2¼ | 2 | 5 |
| VII | Lard | 18. xi. 35 | 0 | ¾ | 1½ | 2¼ | 3 | 5 |
| VIII | Linseed oil | 18. xi. 35 | 0 | ½ | 1 | 1½ | 2 | 6 |
| IX | Cod-liver oil | 9. ii. 36 | 0 | 3/16 | 6/16 | 9/16 | 3 | 5 |
| X | Cod-liver oil | 9. iii. 36 | 0 | 3/16 | 6/16 | 9/16 | 2 | 5 |
| XI | Whale oil | 26. x. 36 | 0 | 3/16 | 6/16 | 9/16 | 2 | 5 |
| XII | Soya-bean oil | 23. xi. 36 | 0 | ½ | 1 | 1½ | 2 | 5 |
| XIII | Soya-bean oil | 4. i. 37 | 0 | 3/10 | 6/10 | 9/10 | 2 and 3 | 10 |
| XIV | Palm oil | 9. iii. 36 | 0 | ½ | 1 | 1½ | 2 | 5 |
| XV | Palm oil | 16. iii. 36 | 0 | ½ | 1· | 1½ | 3 | 5 |
| XVI | Palm oil | 26. x. 36 | 0 | ½ | 1 | 1½ | 3 | 10 |
| XVII | Palm oil | 2. xi. 36 | 0 | ½ | 1 | 1½ | 3 | 20 |

*Notes on Table III*

*Exp. VIII.* An experiment was commenced with a maximum fat feeding of 2¼ lb. daily, but as no cow would consume that amount, the experiment was recommenced with the treatments shown in the table.

*Exp. IX.* Udder trouble developed in one cow in this experiment, and therefore figures had to be calculated, by the missing plot technique, for 1 cow-week.

*Exp. XI.* Preliminary trial showed that 9 oz. of whale oil per day was the maximum that cows would consume.

*Exp. XIII.* One cow dried off prematurely, and figures had to be calculated for each of her last two periods; it was fortunate that the necessity of calculating 2 plots only arose in this 5 × 5 square. The fifth treatment was 1 $\frac{2}{10}$ lb. of oil daily.

*Exp. XIV.* One cow dried off prematurely, and figures had to be calculated for her final period.

butterfat percentages of the milk, with which no effort was made to obtain a group of cows of the same level.

In all except one experiment the cows were on full winter rations, and though the composition of these varied somewhat from time to time the oil content of the concentrated part of the ration only varied from 0·027 to 0·037 lb. per lb. In addition to concentrates, the cows received hay, together with sugar-beet pulp or kale. The total daily intake of oil varied somewhat according to the yield of a cow, and consequent variation in amount of cake fed. In no case did it fall below 0·7 lb. per day, and it never exceeded 1·1 lb. per day. It must be made quite clear that where any fats or oils were fed they were added to the normal balanced rations, and that the amount of oil added was in no way related to the yield of the cow. Where the oil was sufficiently fluid it was merely poured over the weighed out cake ration of the cow, the mixture being then stirred; in some cases, e.g. butter, lard, palm oil, the fat or oil was first warmed sufficiently to make it mix easily.

A summarized description of the experiments is given in Table III.

## RESULTS

As stated earlier, the figures which it is proposed to give are those for the whole of each unit period, although in certain cases, particularly with cod-liver oil, this will tend slightly to lessen the significant differences. In the statistical reduction of the data the procedure has been to work out the results not only with the full daily yield, but also separately for morning, evening, and, where available, noon milkings. In all, the seventeen experiments produced over 500 analyses of variance, and naturally a few significances were obtained with the separate milkings which were not consistent with the results from daily totals; the proportion of such significances was not larger than would be expected through chance, and since there was no general tendency for differences to vary between the milkings, results need only be stated for daily totals. Thus it has been possible to reduce the total data to that presented in Tables IV–VIII, which give, for the variables stated, the results based on the whole unit period.

Table IV shows that the milk yield was not in general affected by the treatments; of the whole seventeen, only one attained significance (that was only with $P < 0.05$) and there is no consistent trend in the actual figures.

The figures for butterfat percentage, shown in Table V, reveal a number of significant differences, and these significances are closely

paralleled by those in Table VI for butterfat yield; it thus appears that, in general, effects on butterfat yield are produced through butterfat percentage, rather than through milk yield.

Table IV. *Summarized results with milk yield*

| Exp. | Oil or fat fed | Relative milk yields for following levels of fat feeding: None | Single | Double | Treble | S.E. of mean | Mean daily milk yield in control periods | Significance |
|---|---|---|---|---|---|---|---|---|
| I | Cotton-seed oil | 100·0 | 103·3 | 104·7 | 102·5 | 1·33 | 24·1 | Insig. |
| II | Cotton-seed oil | 100·0 | 98·5 | 100·7 | 101·2 | 0·85 | 43·7 | Insig. |
| III | Butter | 100·0 | 102·1 | 100·3 | 96·5 | 2·96 | 52·6 | Insig. |
| IV | Butter | 100·0 | 101·9 | 100·1 | 99·8 | 3·49 | 32·9 | Insig. |
| V | Butter | 100·0 | 98·6 | 100·8 | 100·7 | 0·78 | 22·4 | Insig. |
| VI | Lard | 100·0 | 99·3 | 97·2 | 96·1 | 1·38 | 21·1 | Insig. |
| VII | Lard | 100·0 | 101·1 | 98·8 | 99·5 | 1·03 | 42·5 | Insig. |
| VIII | Linseed oil | 100·0 | 101·3 | 100·8 | 96·7 | 2·95 | 18·4 | Insig. |
| IX | Cod-liver oil | 100·0 | 101·8 | 96·3 | 98·3 | 1·75 | 47·1 | Insig. |
| X | Cod-liver oil | 100·0 | 105·6 | 101·6 | 104·4 | 2·09 | 22·5 | Insig. |
| XI | Whale oil | 100·0 | 99·1 | 99·1 | 101·7 | 1·46 | 20·3 | Insig. |
| XII | Soya-bean oil | 100·0 | 98·6 | 105·0 | 105·6 | 4·55 | 14·8 | Insig. |
| XIII | Soya-bean oil | 100·0 | 92·0 | 101·2 | 91·9 | 4·53 | 34·8 | Insig.† |
| XIV | Palm oil | 100·0 | 98·6 | 99·9 | 104·0 | 3·14 | 19·3 | Insig. |
| XV | Palm oil | 100·0 | 100·2 | 101·1 | 100·2 | 2·10 | 39·2 | Insig. |
| XVI | Palm oil | 100·0 | 103·4 | 108·9 | 100·7 | 4·21 | 34·1 | Insig. |
| XVII | Palm oil | 100·0 | 102·4 | 103·6 | 107·9 | 1·47 | 36·6 | Reg.* |

† Relative yield for quadruple fat feeding was 103·0.
* Significant $P<0·05$.

Table V. *Summarized results with butterfat percentage*

| Experiment | Oil or fat fed | Relative butterfat percentages for following levels of fat feeding: None | Single | Double | Treble | S.E. of mean | Mean daily butterfat percentage in control periods | Significance |
|---|---|---|---|---|---|---|---|---|
| I | Cotton-seed oil | 100·0 | 102·9 | 104·6 | 104·0 | 1·99 | 3·79 | Insig. |
| II | Cotton-seed oil | 100·0 | 103·1 | 99·6 | 101·5 | 2·06 | 3·26 | Insig. |
| III | Butter | 100·0 | 105·9 | 112·4 | 117·5 | 2·14 | 3·08 | Reg.** |
| IV | Butter | 100·0 | 92·4 | 98·4 | 106·2 | 5·08 | 3·84 | Insig. |
| V | Butter | 100·0 | 114·3 | 117·1 | 116·1 | 1·87 | 3·40 | Fat > None** |
| VI | Lard | 100·0 | 103·9 | 102·7 | 100·6 | 1·96 | 3·93 | Insig. |
| VII | Lard | 100·0 | 111·4 | 112·8 | 109·1 | 1·47 | 3·19 | Fat > None** |
| VIII | Linseed oil | 100·0 | 103·4 | 102·5 | 108·1 | 2·71 | 3·84 | Insig. |
| IX | Cod-liver oil | 100·0 | 92·8 | 86·5 | 86·7 | 4·22 | 2·93 | Reg.* |
| X | Cod-liver oil | 100·0 | 99·3 | 92·9 | 88·8 | 3·98 | 3·20 | Reg.* |
| XI | Whale oil | 100·0 | 96·3 | 97·5 | 95·8 | 3·15 | 3·87 | Insig. |
| XII | Soya-bean oil | 100·0 | 105·1 | 106·2 | 106·1 | 3·46 | 4·15 | Insig. |
| XIII | Soya-bean oil | 100·0 | 102·5 | 94·6 | 101·1 | 3·23 | 3·74 | Insig.‡ |
| XIV | Palm oil | 100·0 | 110·4 | 112·9 | 111·7 | 2·63 | 3·50 | Fat > None* |
| XV | Palm oil | 100·0 | 109·4 | 103·5 | 111·4 | 3·46 | 3·24 | Insig. |
| XVI | Palm oil | 100·0 | 103·1 | 101·6 | 104·4 | 1·75 | 3·55 | Insig. |
| XVII | Palm oil | 100·0 | 102·7 | 104·8 | 100·1 | 1·48 | 4·03 | Insig. |

* Significant, $P<0·05$.
** Significant, $P<0·01$.
‡ Relative butterfat percentage for quadruple fat feeding was 94·2.

As regards percentage and yield of solids-not-fat, differences were extremely small, but experimental errors were almost microscopic, so that several significances emerged. Little notice can be taken of the significances, however, because the differences involved were too small to be of any practical value.

The methods of sampling described earlier in the paper enabled the true yield of butterfat (and solids-not-fat when studied) to be calculated in every case. Consequently the products of the figures for milk yield and butterfat percentage, shown in Tables IV and V, do not check, in some cases even approximately, with the figures for butterfat yield, shown in Table VI; in general, however, no wide disagreements occur.

Table VI. *Summarized results with butterfat yield*

| Experiment | Oil or fat fed | Relative butterfat yields for following levels of fat feeding: None | Single | Double | Treble | S.E. of mean | Mean daily butterfat yield in control periods | Significance |
|---|---|---|---|---|---|---|---|---|
| I | Cotton-seed oil | 100·0 | 106·3 | 109·7 | 106·4 | 1·84 | 0·91 | Fat > None* |
| II | Cotton-seed oil | 100·0 | 100·5 | 99·6 | 101·5 | 2·62 | 1·44 | Insig. |
| III | Butter | 100·0 | 107·4 | 113·8 | 114·8 | 1·20 | 1·61 | Reg.** |
| IV | Butter | 100·0 | 94·4 | 98·2 | 103·9 | 4·10 | 1·26 | Insig. |
| V | Butter | 100·0 | 113·6 | 117·5 | 116·5 | 2·05 | 0·75 | Fat > None** |
| VI | Lard | 100·0 | 102·7 | 99·8 | 96·0 | 2·50 | 0·84 | Insig. |
| VII | Lard | 100·0 | 111·9 | 111·5 | 108·3 | 1·60 | 1·35 | Fat > None** |
| VIII | Linseed oil | 100·0 | 104·3 | 101·9 | 103·9 | 2·00 | 0·71 | Insig. |
| IX | Cod-liver oil | 100·0 | 93·8 | 81·7 | 83·9 | 6·14 | 1·39 | Reg.* |
| X | Cod-liver oil | 100·0 | 105·2 | 94·5 | 92·5 | 2·23 | 0·75 | Reg.* |
| XI | Whale oil | 100·0 | 94·8 | 94·9 | 97·8 | 4·34 | 0·78 | Insig. |
| XII | Soya-bean oil | 100·0 | 103·2 | 109·6 | 107·2 | 3·96 | 0·61 | Insig. |
| XIII | Soya-bean oil | 100·0 | 96·0 | 94·7 | 93·7 | 3·60 | 1·25 | Insig.‡ |
| XIV | Palm oil | 100·0 | 104·3 | 111·9 | 116·3 | 5·21 | 0·66 | Insig. |
| XV | Palm oil | 100·0 | 110·0 | 103·3 | 110·4 | 2·24 | 1·28 | Fat > None* |
| XVI | Palm oil | 100·0 | 109·5 | 113·2 | 107·0 | 3·68 | 1·19 | Insig. |
| XVII | Palm oil | 100·0 | 106·1 | 109·5 | 109·3 | 2·17 | 1·46 | Reg.* |

* Significant, $P < 0{\cdot}05$.
** Significant, $P < 0{\cdot}01$.
‡ Relative butterfat yield for quadruple fat feeding was 93·2.

Table VII. *Summarized results with solids-not-fat percentage*

| Experiment | Oil or fat fed | Relative solids-not-fat percentage for following levels of fat feeding: None | Single | Double | Treble | S.E. of mean | Mean daily solids-not-fat percentage in control periods | Significance |
|---|---|---|---|---|---|---|---|---|
| XI | Whale oil | 100·0 | 100·2 | 100·1 | 101·3 | 0·82 | 9·35 | Insig. |
| XII | Soya-bean oil | 100·0 | 102·0 | 100·9 | 101·7 | 0·47 | 9·58 | Fat > None* |
| XIII | Soya-bean oil | 100·0 | 102·0 | 100·4 | 101·1 | 0·72 | 9·78 | Insig.† |
| XVI | Palm oil | 100·0 | 99·6 | 101·2 | 100·7 | 1·14 | 9·47 | Insig. |
| XVII | Palm oil | 100·0 | 100·0 | 100·2 | 99·0 | 0·38 | 9·34 | Single and double > treble* |

* Significant, $P < 0{\cdot}05$.
† Relative solids-not-fat percentage for quadruple fat feeding was 101·1.

Table VIII. *Summarized results with solids-not-fat yield*

| Experiment | Oil or fat fed | Relative solids-not-fat yield for following levels of fat feeding: None | Single | Double | Treble | S.E. of mean | Mean daily solids-not-fat yield *in control* periods | Significance |
|---|---|---|---|---|---|---|---|---|
| XI | Whale oil | 100·0 | 99·2 | 98·6 | 103·3 | 1·27 | 1·88 | Insig. |
| XII | Soya-bean oil | 100·0 | 100·4 | 106·6 | 107·6 | 4·62 | 1·41 | Insig. |
| XIII | Soya-bean oil | 100·0 | 94·4 | 101·7 | 93·4 | 3·71 | 3·36 | Insig.† |
| XVI | Palm oil | 100·0 | 103·5 | 110·4 | 101·6 | 4·30 | 3·22 | Insig. |
| XVII | Palm oil | 100·0 | 102·6 | 104·0 | 107·1 | 1·55 | 3·41 | Reg.* |

† Relative solids-not-fat yield for quadruple fat feeding was 103·8.
* Significant, $P < 0·05$.

The results with the various fats and oils may be summarized:

1. *Cod-liver oil* definitely and seriously decreased butterfat percentage, and hence butterfat yield, without affecting milk yield. The highest amount fed per cow daily was only 9 oz., and the true deleterious effect, as stated earlier, has probably been underestimated.

2. *Whale oil* was very unpalatable, and owing to the difficulty in persuading cows to eat it only one experiment with it was completed; no significant effects emerged, but actual differences were unfavourable to the oil.

3. *Linseed oil* was not only unpalatable, but tended to make the cows scour, and again only one experiment was completed. The results were quite insignificant, though the actual differences favoured the oil.

4. *Soya-bean oil* affected neither milk yield not butterfat percentage, but in one of the two experiments it produced a significant increase in the solids-not-fat percentage of the milk; this last difference, however, was very small and was only significant because of an extremely low experimental error.

5. *Cotton-seed oil* produced a significant increase in fat yield in one of two experiments, the increase being due partly to higher milk yields and partly to higher butterfat percentages. Differences with this oil were small but in general favoured it.

6. *Lard* proved palatable, and produced significant and appreciable increases in butterfat precentages and butterfat yield in one of two experiments.

7. *Butter* had no effect on milk yield, but in two of the three experiments it produced large, and highly significant, increases in butterfat percentage, and hence in butterfat yield.

8. *Palm oil* was tested in four experiments of varying lengths, and generally produced an increase in butterfat percentage and yield; the

increase in butterfat yield was significant in two of the experiments, the increase in one case of a short-term experiment being produced by an increase in butterfat percentage, and in the other case by increase in milk yield. In addition, one significance emerged in connexion with solids-not-fat percentage, but the difference involved was exceedingly minute.

From the above it appears that palm oil, butter, lard and possibly cotton-seed oil may generally be expected to increase the butterfat yield of a cow; soya-bean, linseed and whale oils appear to have no appreciable effect in this respect; cod-liver oil very definitely decreases the butterfat percentage of the milk.

The actual oils used in the experiments were not chemically investigated, but in Table IX typical analyses of the oils here used have been

Table IX. *Percentages of fatty acids in typical samples of oils*

| | Butter (1) | Palm oil (2) | Lard (3) | Cotton-seed oil (4) | Linseed oil (5) | Soya-bean oil (5) | Whale oil (6) | Cod-liver oil (7) |
|---|---|---|---|---|---|---|---|---|
| Butyric | 3·5 | — | — | — | — | — | — | — |
| Caproic | 1·7 | — | — | — | — | — | — | — |
| Caprylic | 1·3 | — | — | — | — | — | — | — |
| Capric | 3·1 | — | — | — | — | — | — | — |
| Lauric | 4·1 | — | — | — | — | — | — | — |
| Myristic | 11·1 | 1·2 | 3·96 | 0·5 | — | — | 4·5 | 5·5 |
| Palmitic | 27·3 | 39·6 | 27·66 | 22·0 | 6·2 | 6·4 | 11·5 | 10·5 |
| Stearic | 11·5 | 5·8 | 17·56 | 2·0 | 3·0 | 4·4 | 2·5 | — |
| Saturated acids | 63·6 | 46·6 | 49·18 | 24·5 | 9·2 | 10·8 | 18·5 | 16·0 |
| Palmitoleic | — | — | — | — | — | — | 17·0 | 17·0 |
| Arachidic | 0·6 | — | — | — | — | — | — | — |
| Oleic | 31·3 | 42·4 | 35·64 | 30·5 | 11·4 | 29·6 | 36·5 | 28·5 |
| Linoleic | 4·5 | 11·0 | 13·66 | 45·0 | 39·0 | 52·6 | — | |
| Linolenic | — | — | — | — | 40·4 | 7·0 | — | — |
| $C_{20}$–$C_{24}$ unsaturated acids | — | — | 1·41 | — | — | — | 27·5 | 39·5 |
| Unsaponifiable matter | — | — | 0·11 | — | — | — | 0·5 | — |

(The cod-liver oil value 28·5 is bracketed for Oleic and Linoleic together.)

(1) Hilditch & Shightholme (1930).
(2) Hilditch & Dean (1933). Figures are for oils from Lagos, which most closely corresponded with that used in these experiments in iodine and acid values.
(3) Banks & Hilditch (1932).
(4) Bhattacharya & Hilditch (1931).
(5) Cruickshank (1938).
(6) Thorpe (1927).
(7) Drummond & Hilditch (1930).

assembled from the literature. From this table it is at once clear that the oils which have produced favourable results are those which have high proportions of saturated acids in their fatty acids. Thus, butter, palm oil and lard are very high in this respect; linseed, soya-bean, whale and

cod-liver oil, are low, whilst cotton-seed oil takes an intermediate position.

In the whole series of seventeen experiments, twenty-one different cows were used, some being included in as many as six separate experiments. There was of course some variation in the response of different individual cows to fat or oil feeding; the most glaring case occurred in Exp. IV. In this case three cows gave a marked increase in butterfat percentage when butter was added to the ration, but with the fourth the butterfat percentage decreased markedly when butter was added; this explains the inconsistency shown above, that the second experiment with butter was insignificant, whilst the first and third were highly significant. It was interesting to observe that this individual peculiarity does not appear to persist, because this particular cow was included in five other experiments, where she conformed to other animals in behaviour; she was apparently perfectly healthy in all experiments, including that one in which she behaved peculiarly.

## Summary

1. A description is given of an adaptation of the Latin square lay-out to experiments with dairy cows. Experiments were conducted to test the effect of adding various fats and oils to the ration, and since effects were rapidly produced ,the Latin square technique proved very efficient, experimental errors being rather lower than those usually obtained in experiments with crops.

2. Seventeen separate experiments, including, in all, twenty-one different cows (many of whom were used more than once), were conducted; fourteen of these were short-term experiments with unit periods of 5 days, two were medium-term with unit periods of 10 days and one long-term with 20 days.

3. Palm oil, butter, lard and possibly cotton-seed oil were found to increase butterfat yield, chiefly by raising the butterfat percentage of the milk. Soya-bean, linseed and whale oils were without effect. Cod-liver oil definitely decreased butterfat percentage and butterfat yield.

4. It appears that the beneficial oils are those containing a large proportion of the saturated fatty acids.

5. Experiments with the same oil were not always consistent, and it appears that the effect may vary from cow to cow, and also with the same cow at different times.

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# THE ANALYSIS OF GROUPS OF EXPERIMENTS

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(With One Text-figure)

## 1. Introduction

Agricultural experiments on the same factor or group of factors are usually carried out at a number of places and repeated over a number of years. There are two reasons for this. First, the effect of most factors (fertilizers, varieties, etc.) varies considerably from place to place and from year to year, owing to differences of soil, agronomic practices, climatic conditions and other variations in environment. Consequently the results obtained at a single place and in a single year, however accurate in themselves, are of limited utility either for the immediate practical end of determining the most profitable variety, level of manuring, etc., or for the more fundamental task of elucidating the underlying scientific laws. Secondly, the execution of any large-scale agricultural research demands an area of land for experiment which is not usually available at a single experimental station, and consequently much experimental work is conducted co-operatively by farmers and agricultural institutions which are not themselves primarily experimental.

The agricultural experimenter is thus frequently confronted with the results of a set of experiments on the same problem, and has the task of analysing and summarizing these, and assigning standard errors to any estimates he may derive. Though at first sight the statistical problem (at least in the simpler cases) appears to be very similar to that of the analysis of a single replicated trial, the situation will usually on investigation be found to be more complex, and the uncritical application of methods appropriate to single experiments may lead to erroneous conclusions. The object of this paper is to give illustrations of the statistical procedure suitable for dealing with material of this type.

## 2. General considerations

Agronomic experiments are undertaken with two different aims in view, which may roughly be termed the technical and the scientific. Their aim may be regarded as scientific in so far as the elucidation of the under-

lying laws is attempted, and as technical in so far as empirical rules for the conduct of practical agriculture are sought. The two aims are, of course, not in any sense mutually exclusive, and the results of most well-conducted experiments on technique serve to add to the structure of general scientific law, or at least to indicate places where the existing structure is inadequate, while experiments on questions of a more fundamental type will themselves provide the foundation of further technical advances.

In so far as the object of a set of experiments is technical, the estimation of the average response to a treatment, or the average difference between varieties, is of considerable importance even when this response varies from place to place or from year to year. For unless we both know the causes of this variation and can predict the future incidence of these causes we shall be unable to make allowance for it, and can only base future practice on the average effects. Thus, for example, if the response to a fertilizer on a certain soil type and within a certain district is governed by meteorological events subsequent to its application the question of whether or not it is profitable to apply this fertilizer, and in what amount, must (in the absence of any prediction of future meteorological events) be governed by the average response curve over a sufficiently representative sample of years. In years in which the weather turns out to be unfavourable to the fertilizer a loss will be incurred, but this will be compensated for by years which are especially favourable to the fertilizer.

Any experimental programme which is instituted to assess the value of any particular treatment or practice or to determine the optimal amount of such treatment should therefore be so designed that it is capable of furnishing an accurate and unbiased estimate of the average response to this treatment in the various combinations of circumstances in which the treatment will subsequently be applied. The simplest and indeed the only certain way of ensuring that this condition shall be fulfilled is to choose fields on which the experiments are to be conducted by random selection from all fields which are to be covered by the subsequent recommendations.

The fact that the experimental sites are a random sample of this nature does not preclude different recommendations being made for different categories included in this random sample. We may, for instance, find that the response varies according to the nature of the previous crop, in which case the recommendations may be correspor lingly varied. Moreover, in a programme extending over several years, the recommendations may become more specific as more information is accumulated, and

the experiments themselves may be used to determine rules for the more effective application of the treatments tested, as in fertilizer trials in which the chemical examination of soil samples may lead to the evolution of practical chemical tests for fertilizer requirements.

*At present it is usually impossible to secure a set of sites selected* entirely at random. An attempt can be made to see that the sites actually used are a "representative" selection, but averages of the responses from such a collection of sites cannot be accepted with the same certainty as would the averages from a random collection.

On the other hand, comparisons between the responses on different sites are not influenced by lack of randomness in the selection of sites (except in so far as an estimate of the variance of the response is required) and indeed for the purpose of determining the exact or empirical natural laws governing the responses, the deliberate inclusion of sites representing extreme conditions may be of value. Lack of randomness is then only harmful in so far as it results in the omission of sites of certain types and in the consequent arbitrary restriction of the range of conditions. In this respect scientific research is easier than technical research.

### 3. The analogy between a set of experiments and a single replicated trial

If a number of experiments containing the same varieties (or other treatments) are carried out at different places, we may set out the mean yields of each variety at each place in the form of a two-way table. The marginal means of this table will give the average differences between varieties and between places. The table bears a formal analogy to the two-way table of individual plot yields, arranged by blocks and varieties, of a randomized block experiment, and we can therefore perform an analysis of variance in the ordinary manner, obtaining a partition of the degrees of freedom (in the case of six places and eight varieties, for example) as follows:

| | Degrees of freedom |
|---|---|
| Places | 5 |
| Varieties | 7 |
| Remainder | 35 |
| Total | 47 |

The remainder sum of squares represents that part of the sum of squares which is due to variation (real or apparent) of the varietal differences at the different places. This variation may reasonably be called the *interaction* between varieties and places. It will include a component

of variation arising from the experimental errors at the different places.

If the experiments are carried out in randomized blocks (or in any other type of experiment allowing a valid estimate of error) the above analysis may be extended to include a comprehensive analysis of the yields of the individual plots. If there are five replicates at each place, for example, there will be 240 plot yields, and the partition of the degrees of freedom will then be as follows:

| | Degrees of freedom |
|---|---|
| Places | 5 |
| Varieties | 7 |
| Varieties × places | 35 |
| Blocks | 24 |
| Experimental error | 168 |
| Total | 239 |

It should be noted that in this analysis the sums of squares for varieties and for varieties × places are together equal to the total of the sums of squares for varieties in the analyses of the separate experiments. Similarly the sums of squares for blocks and for experimental error are equal to the totals of these items in the separate analyses. If, as is usual, the comprehensive analysis is given in units of a single plot yield, the sums of squares derived from the two-way table of places and varieties must be multiplied or divided by 5 according as means or totals are there tabulated.

The first point to notice about this comprehensive analysis of variance is that the estimates of error from all six places are pooled. If the errors of all experiments are substantially the same, such pooling gives a more accurate estimate than the estimates derived from each separate experiment, since a larger number of degrees of freedom is available. If the errors are different, the pooled estimate of the error variance is in fact the estimate of the mean of the error variances of the separate experiments. It will therefore still be the correct estimate of the error affecting the mean difference (over all places) of two varieties, but it will no longer be applicable to comparisons involving some of the places only. Moreover, as will be explained in more detail below, the ordinary tests of significance, even of means over all places, will be incorrect.

If the errors of all the experiments are the same, the other mean squares in the analysis of variance table may be compared with the mean square for experimental error by means of the $z$ test. The two comparisons of chief interest are those for varieties and for varieties × places. The meaning of these will be clear if we remember that there is a separate set of varietal means at each place, and that the differences between these

means are not necessarily the same at all places. If the mean square for varieties is significant, this indicates the significance of the average differences of the varieties *over the particular set of places chosen.* If varieties × places is also significant, a significant variation from place to place in the varietal differences is indicated. In this latter case it is clear that the choice of places must affect the magnitude of the average differences between varieties: with a different set of places we might obtain a substantially different set of average differences. Even if varieties × places is not significant, this fact cannot be taken as indicating *no* variation in the varietal differences, but only that such variation is likely to be smaller than an amount which can be determined by the arguments of fiducial probability.

We may, therefore, desire to determine the variation that is likely to occur in the average differences between the varieties when different sets of places are chosen, and in particular whether the average differences actually obtained differ significantly from zero when variation from place to place is allowed for. Endless complications affect this question, and with the material ordinarily available a definite answer is usually impossible. The various points that arise will be made clear by an actual example, but first we may consider the situation in the ideal case where the chosen places are a strictly random selection from all possible places.

At first sight it would appear to be legitimate in this case to compare the mean square for varieties with that for varieties × places by means of the $z$ test. There is, however, no reason to suppose that the variation of the varietal differences from place to place is the same for each pair of varieties. Thus the eight varieties of our example might consist of two sets of four, the varieties of each set being closely similar among themselves but differing widely from those of the other set, not only in their average yield, but also in their variations in yield from place to place.

The sums of squares for varieties and for varieties × places would then have large components derived from one degree and five degrees of freedom respectively, while the remaining components might be of the order of experimental error. In the limiting case, therefore, when the experimental error is negligible in comparison with the differences of the two sets and the average difference over all possible places is zero, the $z$ derived from the two mean squares will be distributed as $z$ for 1 and 5 degrees of freedom instead of as $z$ for 7 and 35 degrees of freedom. Verdicts of significance and of subnormal variation will therefore be reached far more often than they should be.

The correct procedure in this case is to divide the sums of squares for

varieties and for varieties × places into separate components, and compare each component separately. Thus we shall have:

| | Degrees of freedom |
|---|---|
| Varieties: Sets | 1 |
| Within sets | 6 |
| Varieties × places: Sets | 5 |
| Within sets | 30 |

The 1 degree of freedom between sets can now legitimately be compared with the 5 degrees of freedom for sets × places, but the degrees of freedom within sets may require further subdivision before comparison.

It is worth noting that the test of a single degree of freedom can be made by the $t$ test, by tabulating the differences between the means of the two sets for each place separately. This test is in practice often more convenient than the $z$ test, of which it is the equivalent.

## 4. An actual example of the analysis of a set of variety trials

Table I, which has been reproduced by Fisher (1935) as an example for analysis by the reader, gives the results of twelve variety trials on barley conducted in the State of Minnesota and discussed by Immer *et al.* (1934). The trials were carried out at six experiment stations in each of two years, and actually included ten varieties of which only five (those selected by Fisher) are considered here.

Table I. *Yields of barley varieties in twelve independent trials. Totals of three plots, in bushels per acre*

| Place and year | Manchuria | Svansota | Velvet | Trebi | Peatland | Total |
|---|---|---|---|---|---|---|
| University Farm 1931 | 81·0 | 105·4 | 119·7 | 109·7 | 98·3 | 514·1 |
| 1932 | 80·7 | 82·3 | 80·4 | 87·2 | 84·2 | 414·8 |
| Waseca 1931 | 146·6 | 142·0 | 150·7 | 191·5 | 145·7 | 776·5 |
| 1932 | 100·4 | 115·5 | 112·2 | 147·7 | 108·1 | 583·9 |
| Morris 1931 | 82·3 | 77·3 | 78·4 | 131·3 | 89·6 | 458·9 |
| 1932 | 103·1 | 105·1 | 116·5 | 139·9 | 129·6 | 594·2 |
| Crookston 1931 | 119·8 | 121·4 | 124·0 | 140·8 | 124·8 | 630·8 |
| 1932 | 98·9 | 61·9 | 96·2 | 125·5 | 75·7 | 458·2 |
| Grand Rapids 1931 | 98·9 | 89·0 | 69·1 | 89·3 | 104·1 | 450·4 |
| 1932 | 66·4 | 49·9 | 96·7 | 61·9 | 80·3 | 355·2 |
| Duluth 1931 | 86·9 | 77·1 | 78·9 | 101·8 | 96·0 | 440·7 |
| 1932 | 67·7 | 66·7 | 67·4 | 91·8 | 94·1 | 387·7 |
| **Total** | 1132·7 | 1093·6 | 1190·2 | 1418·4 | 1230·5 | 6065·4 |

The experiments were all arranged in randomized blocks with three replicates of each variety. When all ten varieties are included there are therefore 18 degrees of freedom for experimental error at each station.

The error mean squares for the twelve experiments were computed

from the yields of the separate plots, which have been given in full by Immer. They are shown in Table II.

Table II. *Error mean squares of barley experiments*

| | Mean square | | Approximate $\chi^2$ | |
|---|---|---|---|---|
| | 1931 | 1932 | 1931 | 1932 |
| University Farm | 21·25 | 15·98 | 16·43 | 12·36 |
| Waseca | 26·11 | 25·21 | 20·19 | 19·49 |
| Morris | 18·62 | 20·03 | 14·40 | 15·49 |
| Crookston | 30·27 | 21·95 | 23·40 | 16·97 |
| Grand Rapids | 26·28 | 26·40 | 20·32 | 20·41 |
| Duluth | 27·00 | 20·28 | 20·88 | 15·68 |

If the errors at all stations are in fact the same, these error mean squares, when divided by the true error variance and multiplied by the number of degrees of freedom, 18, will be distributed as $\chi^2$ with 18 degrees of freedom. If we take the mean of the error mean squares, 23·28, as an estimate of the true error variance, the distribution obtained will not be far removed from the $\chi^2$ distribution. The actual values so obtained are shown in Table II, and their distribution is compared with the $\chi^2$ distribution in Table III. Variation in the experimental error from station to station would be indicated by this distribution having a wider dispersion than the $\chi^2$ distribution.

Table III. *Comparison with the theoretical $\chi^2$ distribution*

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P$ | 1·0 | 0·99 | 0·98 | 0·95 | 0·90 | 0·80 | 0·70 | 0·50 | 0·30 | 0·20 | 0·10 | 0·05 | 0·02 | 0·01 | 0 |
| $\chi^2$ | 0 | 7·02 | 7·91 | 9·39 | 10·86 | 12·86 | 14·44 | 17·34 | 20·60 | 22·76 | 25·99 | 28·87 | 32·35 | 34·80 | ∞ |
| No. observed | 0 | 0 | 0 | 0 | 1 | 1 | 4 | 4 | 1 | 1 | 0 | 0 | 0 | 0 | |
| No. expected | 0·12 | 0·12 | 0·36 | 0·6 | 1·2 | 1·2 | 2·4 | 2·4 | 1·2 | 1·2 | 0·36 | 0·36 | 0·12 | 0·12 | |

In this case it is clear that the agreement is good, and consequently we shall be doing no violence to the data if we assume that the experimental errors are the same for all the experiments. This gives 23·28 as the general estimate (216 degrees of freedom) for the error variance of a single plot, and the standard error of the values of Table I is therefore $\sqrt{(3 \times 23{\cdot}28)}$ or $\pm 8{\cdot}36$.

The analysis of variance of the values of Table I is given in Table IV in units of a single plot. The components due to places and years have been separated in the analysis in the ordinary manner.

Every mean square, except that for varieties × years, will be found, on testing by the $z$ test, to be significantly above the error mean square. Examination of Table I indicates that variety Trebi is accounting for a good deal of the additional variation due to varieties and varieties × places, for the mean yield of this variety over all the experiments is much

above that of the other four varieties, but at University Farm and Grand Rapids, two of the lowest yielding stations, it has done no better than the other varieties.

Table IV. *General analysis of variance (units of a single plot)*

| | Degrees of freedom | Sum of squares | Mean square | |
|---|---|---|---|---|
| Places | 5 | 7073·64 | 1414·73 | |
| Years | 1 | 1266·17 | 1266·17 | |
| Places × years | 5 | 2297·96 | 459·59 | |
| Varieties | 4 | 1769·99 | 442·50 | |
| Varieties × places | 20 | 1477·67 | 73·88 | |
| Varieties × years | 4 | 97·27 | 24·32 | 42·72 |
| Varieties × places × years | 20 | 928·09 | 46·40 | |
| Total | 59 | 14910·79 | | |
| Experimental error | 216 | | 23·28 | |

In order to separate the effect of Trebi it is necessary to calculate the difference between the yield of Trebi and the mean of the yields of the other four varieties for each of the twelve experiments, and to analyse the variance of these quantities. For purposes of computation the quantities:

$$5 \times \text{yield of Trebi} - \text{total yield of station} = 4\ (\text{yield of Trebi} - \text{mean of other varieties})$$

are more convenient.

The analysis of variance is similar to that which gives places, years and places × years in the main analysis. The divisor of the square of a single quantity is $3 \times (4^2+1+1+1+1) = 60$ and the square of the total of all twelve quantities, divided by 720, gives the sum of squares representing the average difference between Trebi and the other varieties over all stations.

The four items involving varieties in the analysis of variance are thus each split up into two parts, representing the difference of Trebi and the other varieties, and the variation of these other varieties among themselves. This partition is given in Table V. The second part of each sum of

Table V. *Analysis of variance, Trebi v. Remainder*

| | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|
| Varieties: Trebi | 1 | 1463·76 | 1463·76 |
| Remainder | 3 | 306·23 | 102·08 |
| Varieties × places: Trebi | 5 | 938·09 | 187·62 |
| Remainder | 15 | 539·58 | 35·97 |
| Varieties × years: Trebi | 1 | 7·73 | 7·73 |
| Remainder | 3 | 89·54 | 29·85 |
| Varieties × places × years: Trebi | 5 | 162·10 | 32·42 |
| Remainder | 15 | 765·97 | 51·06 |

squares can be derived by subtraction, or by calculating the sum of squares of the deviations of the four remaining varieties from their own mean.

Study of this table immediately shows that the majority of the variation in varietal differences between places is accounted for by the difference of Trebi from the other varieties. The mean square for varieties × places has been reduced from 73·88 to 35·97 by the elimination of Trebi, and this latter is in itself not significantly above the experimental error. The mean square for varieties × places × years has not been similarly reduced, however, in fact it is actually increased (though not significantly), and the last three remainder items taken together are still significantly above the experimental error. There is thus still some slight additional variation in response from year to year and place to place. The place to place variation appears to arise about equally from all the remaining three varieties, but the differences between the different years are almost wholly attributable to the anomalous behaviour at Grand Rapids of Velvet, which yielded low in 1931 and high in 1932. If the one degree of freedom from varieties × years and varieties × places × years arising from this difference is eliminated by the "missing plot" technique (Yates, 1933) we have

| | Degrees of freedom | Sum of squares | Mean squares |
|---|---|---|---|
| Velvet at Grand Rapids | 1 | 453·19 | 453·19 |
| Remainder | 23 | 572·16 | 24·88 |

Thus the remainder is all accounted for by experimental error.

It has already been noted that Trebi yielded relatively highly at the high-yielding centres. The degree of association between varietal differences and general fertility (as indicated by the mean of all five varieties) can be further investigated by calculating the regressions of the yields of the separate varieties on the mean yields of all varieties. The deviations of the mean yields of the six stations from the general mean in the order given are nearly proportional to

$$-2,\ +10,\ +1,\ +2,\ -6,\ -5.$$

The sum of the squares of these numbers is 170. Multiplying the varietal totals at each place by these numbers we obtain the sums

| | |
|---|---|
| Manchuria | 1004·6 |
| Svansota | 1196·2 |
| Velvet | 1137·8 |
| Trebi | 1926·8 |
| Peatland | 736·3 |
| Total | 6001·7 |

The sum of the squares of the deviations of these sums, divided by $170 \times 6$, gives the part of the sum of squares accounted for by the differences of the regressions. This can be further subdivided as before, giving:

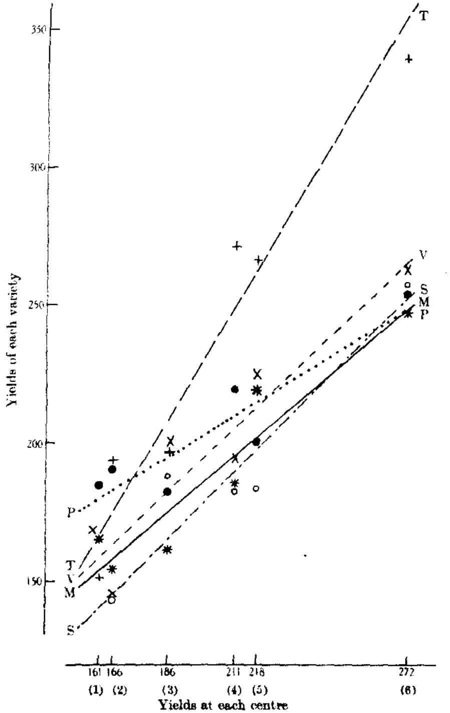
| | | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|---|
| Varieties × Places: | | | | |
| Differences of regressions: | Trebi | 1 | 646·75 | 646·75 |
| | Remainder | 3 | 123·17 | 41·06 |
| | Total | 4 | 769·92 | |
| Deviations from regressions: | Trebi | 4 | 291·34 | 72·84 |
| | Remainder | 12 | 416·39 | 34·70 |
| | Total | 16 | 707·73 | |

Thus the greater part of the differences between Trebi and the remaining varieties is accounted for by a linear regression on mean yield. There is still a somewhat higher residual variation (M.S. 72·84) of Trebi from its own regression than of the other varieties from their regressions, though the difference is not significant. Of the four remaining varieties Peatland appears to have a lower regression than the others, giving significantly higher yields at the lower yielding stations only, the difference in regressions being significant when Peatland is tested against the other three varieties.

The whole situation is set out in graphical form in Fig. 1, where the stations are arranged according to their mean yields and the calculated regressions are shown. It may be mentioned that of the remaining five varieties not included in the discussion, three show similar regressions on mean yield.

This artifice of taking the regression on the mean yield of the difference of one variety from the mean of the others is frequently of use in revealing relations between general fertility and varietal differences. A similar procedure can be followed with response to fertilizers or other treatments. The object of taking the regression on the mean yield rather than on the yield of the remaining varieties is to eliminate a spurious component of regression which will otherwise be introduced by experimental errors. If the variability of each variety at each centre is the same, apart from the components of variability accounted for by the regressions, the regression so obtained will give a correct impression of the results. This is always the situation as far as experimental error is concerned (except in those rare cases in which one variety is more variable than the others). It may or may not be the case for the other components of variability. In our example, as we have seen, the deviations of Trebi from its regression are somewhat greater than those of the other varieties. In such cases we

should theoretically take regressions on a weighted mean yield, but there will be little change in the results unless the additional components of variance are very large.




(1) Grand Rapids, (2) Duluth, (3) University Farm, (4) Morris, (5) Crookston, (6) Waseca

Trebi + ——— Velvet × – – – – Svansota ○ —·—·— Manchuria ✱ ——— Peatland ● ·········

Fig. 1. Regressions on mean yield. The yields shown are totals of the two years. The mean yields per plot (bushels per acre) are ¼ of these totals.

We can also examine how far it is possible to make practical recommendations from the results of these experiments.[1] The following points are of importance:

(1) How far is the superiority of Trebi over the remaining four varieties likely to recur in future years at the same set of stations?

(2) How far is Trebi likely to be superior to the other varieties at other stations in years similar to 1931 and 1932, and in particular with what degree of confidence may Trebi be recommended for general adoption in preference to the other four varieties in some or all of the districts of the state of Minnesota?

The answer to question (1) is clearly limited by the fact that only 2 years' results are available. Therefore, we cannot make any general statement as regards years which are radically different in weather conditions to 1931 and 1932. If, however, 1931 and 1932 themselves differed considerably as regards weather conditions, and if, moreover, the weather conditions varied considerably from station to station in the same year, the weather conditions over the twelve experiments might be regarded as an approximation to a random sample from all possible conditions, in which case the pooled estimate of varieties × years and varieties × places × years (these not being significantly different) might be regarded as an appropriate estimate of the variance due to weather conditions, to differences between fields at the same station, and to experimental error. In this respect Trebi is no more variable than the remaining varieties, and if the anomalous variation of Velvet at Grand Rapids be excluded the pooled estimate (23 degrees of freedom) is only slightly above experimental error. In default of any special explanation of this anomalous variation, however, it will be more reasonable not to exclude this degree of freedom, in which case we should assess the variance due to the above causes as 42·72 (24 degrees of freedom) and this agrees closely with the similar estimate (which cannot be attributed to any one outstanding difference) from the varieties which have been omitted from the present analysis.

Since three plots go to make up the total at any one place in one year, the additional variance of a single varietal mean (on a single plot basis) due to weather conditions and differences between fields at the same station, over that arising from experimental error, is

$$\tfrac{1}{3}\,(42{\cdot}72 - 23{\cdot}28) = 6{\cdot}48,$$

[1] It should be noted that in practice there are many other factors besides yield which must be taken into consideration. In this instance Dr Immer informs me that the malting quality of Trebi is poor.

and the variance of the difference of two varieties due to these causes will be double this, i.e. 12·96.

In addition to this real variation in subsequent years of the varietal differences at a place, the errors of the estimates obtained from the 2 years' experimental results must also be taken into account. These are calculated in the ordinary manner from the mean square, 42·72, for *varieties × years, and varieties × places × years. Thus the error variance* of the estimate of the difference of two varieties at any one place is twice $\frac{1}{6}$ (42·72) = 14·24. (If varieties × years and varieties × places × years were different further subdivision of the components, similar to that illustrated below when considering place to place variation, would be necessary.)

In one sense, therefore, the variance of the expected difference of any two varieties, say of Trebi and Peatland at Waseca, in any subsequent year (*with similar weather conditions*) is 12·96 + 14·24 = 27·20, but it must be remembered that if a whole series of subsequent years is taken the actual differences will not be distributed about the estimated difference 14·2 with variance 27·20, but about some uuknown "true" difference with variance 12·96, the unknown true difference having itself a fiducial distribution about the estimated difference given by the variance 14·24.

The answer to the second question depends on how far the actual stations may be regarded as a random sample of all stations. If this is the case, the estimate of varieties × places for Trebi will be the appropriate estimate of the variance from place to place, including one-half the variance due to differences between fields at the same station, to experimental error and to weather conditions except in so far as they are constant over all stations in each year. This is based on only five degrees of freedom and is therefore ill determined, but accepting the value 187·62, the variance of the difference of Trebi and the remaining varieties due to places only is:

$$\tfrac{1}{6}\,(1+\tfrac{1}{4})\,(187{\cdot}62-42{\cdot}72)=30{\cdot}19,$$

and, therefore, that due to place, field and weather conditions (but *excluding experimental error*) is

$$30{\cdot}19+(1+\tfrac{1}{4})\,6{\cdot}48=38{\cdot}29.$$

In addition to this variation the error of the estimated mean difference must be taken into account. The mean difference of Trebi from the mean of the other varieties is 7·1 per plot, and this has an estimated variance due to places, fields, differences in weather conditions from place to place, and experimental error, of

$$\tfrac{1}{36}\,(1+\tfrac{1}{4})\,187{\cdot}62=6{\cdot}51,$$

and also an additional undetermined component of variance due to differences between years.

Hence, the difference to be expected in a single field is subject to a variation about the true mean having an estimated variance of 38·29, and the estimate of the true mean 7·1 has an error variance of 6·51. Consequently it will frequently happen in default of other information that Trebi will yield less than some other variety that might have been grown. At Grand Rapids, Trebi yielded 15–20% less than Peatland in the 2 years of the experiment. It is poor consolation to the farmer of a farm similar to this to be told that Trebi is giving substantially higher yields on other farms.

It would be rash, however, to recommend Peatland for the whole of the Grand Rapids and Duluth districts and Trebi for the whole of the other districts, in particular the Waseca district, until we know how far variation in the varietal differences depends on factors common to the whole of a district or soil type and how far on factors exclusive to individual farms, such as variations in manuring and cultivation practices and differing crop rotations. Only parallel experiments in the same district on farms which may themselves be reasonably regarded as a random selection from all farms in the district will separate these two sources of variation, and it is therefore impossible from the general analysis of variance to say with any confidence whether Trebi is particularly suited to the district of Waseca or whether its high yield here is due to special conditions at the experimental station. As we have seen, however, the superiority of Trebi is associated with high general level of fertility. If, therefore, we know that the Waseca district is as a whole high yielding we may confidently recommend Trebi for general adoption in the district (with a reservation as to weather conditions). On the other hand, if the general yield of the district is only average, the experimental station being outstanding, then we should only be justified in recommending Trebi for farms of high fertility in this district but might also include farms of high fertility in other districts.

Immer does not report the soil types of the various stations, but it is noteworthy that Peatland, which proved the best variety at the low-yielding stations, has (as its name implies) been specially selected for peat soils, which are likely to be low yielding.

## 5. Example of variation in experimental error

If the experimental errors of the different experiments are substantially different the use of the $z$ test in conjunction with the pooled estimate of error may be misleading, in just the same way as the pooling

of all the degrees of freedom from varieties × places was misleading in the set of varietal trials already considered.

The following is an example in which the $z$ test indicates an almost significant interaction between a treatment effect and places, whereas proper tests show that there is no indication of any such variation in the treatment effect. The example is particularly interesting in that on a first examination of the data the results of the $z$ test led the experimenter to draw false conclusions.

The experiments consisted of a series of thirteen 3 × 3 Latin squares, described by Lewis & Trevains (1934) and carried out in order to test the effectiveness of, and difference between, an ammonium phosphate mixture and an ordinary fertilizer mixture on sugar beet. Large responses to the fertilizers were shown in all the experiments. The question arose as to whether there was any significant difference between the two forms of fertilizer.

Table VI. *Experiments on sugar beet*

| Centre | Yields of roots, tons per acre: No fertilizer | Amm. phos. mixture | Ordinary mixture | Mean response | Amm. phos. − ordinary ($x$) | Error mean square per plot ($s^2$) | $\chi^2$ | $t$ |
|---|---|---|---|---|---|---|---|---|
| 1 | 7·44 | 15·69 | 13·75 | +7·28 | +1·94 | 0·8599 | 3·74 | +2·56 |
| 2 | 7·19 | 12·28 | 11·32 | +4·61 | +0·96 | 0·3543 | 1·54 | +1·98 |
| 3 | 10·07 | 13·93 | 13·10 | +3·44 | +0·83 | 0·5329 | 2·32 | +1·39 |
| 4 | 7·74 | 10·97 | 11·89 | +3·69 | −0·92 | 1·1528 | 5·02 | −1·05 |
| 5 | 11·88 | 13·96 | 15·06 | +2·63 | −1·10 | 0·2638 | 1·15 | −2·68 |
| 6 | 11·94 | 14·35 | 14·36 | +2·42 | −0·01 | 1·7249 | 7·51 | −0·01 |
| 7 | 6·20 | 10·27 | 10·02 | +3·94 | +0·25 | 0·4803 | 2·09 | +0·44 |
| 8 | 8·99 | 11·17 | 11·47 | +2·33 | −0·30 | 0·1107 | 0·482 | −1·10 |
| 9 | 9·46 | 12·54 | 12·46 | +3·04 | +0·08 | 0·0184 | 0·0801 | +0·72 |
| 10 | 7·42 | 10·93 | 10·79 | +3·44 | +0·14 | 0·0046 | 0·0200 | +2·53 |
| 11 | 3·70 | 5·46 | 5·38 | +1·72 | +0·08 | 0·0073 | 0·0318 | +1·15 |
| 12 | 9·62 | 12·72 | 13·01 | +3·24 | −0·29 | 0·1920 | 0·836 | −0·81 |
| 13 | 9·47 | 13·53 | 13·72 | +4·16 | −0·19 | 0·2706 | 1·18 | −0·45 |
| Mean | 8·55 | 12·14 | 12·02 | +3·53 | +0·1115 | 0·4594 | 2·00 | |

The yields are shown in Table VI. The mean yields of the two forms of fertilizer over all experiments are practically identical, indicating an absence of any consistent difference between the two forms. The analysis of variance, using a pooled estimate of error from all squares, is given in Table VII.

The $z$ between treatment × centres and error is 0·361, which is almost significant, the 5% point being 0·382. Inspection of the differences between the two forms shows that eight of these are small ($\leq$ 0·30), while the remaining five range numerically from 0·83 to 1·94. These five are all associated with large error mean squares. The values of $t$ for the separate

Table VII. *Analysis of variance of responses to fertilizer and of difference between mixtures (single-plot basis)*

| | Degrees of freedom | Sum of squares | Mean square | $z$ |
|---|---|---|---|---|
| Response to fertilizer: | | | | |
| Mean response | 1 | 324·7605 | 324·7605 | |
| Response × centres | 12 | 45·8462 | 3·8205 | |
| Differences between mixtures: | | | | |
| Mean difference | 1 | 0·2493 | 0·2493 | |
| Difference × centres | 12 | 11·3542 | 0·9462 | 0·361 |
| Error | 26 | 11·9450 | 0·4594 | |

experiments have therefore been calculated and are given in Table VI. These thirteen observed values are compared with the theoretical $t$ distribution for 2 degrees of freedom in Table VIII. The two distributions agree excellently, not one of the values of $t$ being below the 0·1 level of probability. We must conclude, therefore, that there is no evidence from the experiments that the two mixtures behaved differently at any centre.

Table VIII. *Comparison with theoretical $t$ and $\chi^2$ distributions*

| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P$ | 1·0 | | 0·9 | | 0·8 | | 0·7 | | 0·6 | | 0·5 | | 0·4 | | 0·3 | | 0·2 | | 0·1 | | 0·05 | | 0·02 | | 0·01 | | 0 |
| $t$ | 0 | | 0·14 | | 0·29 | | 0·44 | | 0·62 | | 0·82 | | 1·06 | | 1·39 | | 1·89 | | 2·92 | | 4·30 | | 6·96 | | 9·92 | | $\infty$ |
| Expected | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 1·3 | | 0·65 | | 0·39 | | 0·13 | | 0·13 | |
| Observed | | 1 | | 0 | | ½ | | 1½ | | 2 | | 1 | | 2½ | | ½ | | 4 | | 0 | | 0 | | 0 | | 0 | |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $P$ | 1·0 | | 0·99 | | 0·98 | | 0·95 | | 0·90 | | 0·80 | | 0·70 | | 0·50 | | 0·30 | | 0·20 | | 0·10 | | 0·05 | | 0·02 | | 0·01 | | 0 |
| $\chi^2$ | 0 | | 0·0201 | | 0·0404 | | 0·103 | | 0·211 | | 0·446 | | 0·713 | | 1·39 | | 2·41 | | 3·22 | | 4·60 | | 5·99 | | 7·82 | | 9·21 | | $\infty$ |
| Expected | | 0·13 | | 0·13 | | 0·39 | | 0·65 | | 1·3 | | 1·3 | | 2·6 | | 2·6 | | 1·3 | | 1·3 | | 0·65 | | 0·39 | | 0·13 | | 0·13 | |
| Observed | | 1 | | 1 | | 1 | | 0 | | 0 | | 1 | | 3 | | 3 | | 0 | | 1 | | 1 | | 1 | | 0 | | 0 | |

The above method requires modification if the true difference $\mu$ between the two forms of fertilizer is appreciably different from zero, for the quantities $t' = (x-\mu)/S$ will then conform to the $t$ distribution, instead of the $t$'s calculated as above. The quantity $\mu$ is not exactly known, but if the centres are at all numerous the use of the mean difference $\bar{x}$, or some form of weighted mean difference, such as one of those discussed in the next section, will give quantities which closely approximate to the $t$ distribution.

Although inspection of Table VIII shows quite conclusively in the present example that the observed $t$'s are in no way abnormal, border-line cases will arise in which a proper test of significance is desirable. An obvious form of test would be that based on the variance of the observed $t$'s, or of some analogous function. One such function is the "weighted sum of squares of deviations",

$$Q = Sw\,(x-\bar{x}_w)^2 = Swx^2 - \bar{x}_w \,.\, Swx,$$

where the weights $w$ are the reciprocals of the estimates of the error variances of the differences $x$, and $\bar{x}_w$ is the weighted mean of the $x$'s, i.e.

$$\bar{x}_w = \frac{Swx}{Sw}.$$

If we then calculate

$$\chi'^2 = (k-1) + \sqrt{\frac{n-4}{n-1}} \left\{ \frac{n-2}{n} Q - (k-1) \right\},$$

$\chi'^2$ will be distributed approximately as $\chi^2$ with $k-1$ degrees of freedom. The relation of $Q$ to the ordinary expression for the variance of $t'$ is shown by the alternative form

$$Q = S(t'^2) - \{S(t'\sqrt{w})\}^2/S(w).$$

This test should not be used if $n$ is less than 6. Actual comparison of the distribution of the $t'$'s with the $t$ distribution should then be resorted to, the unweighted mean $\bar{x}$ being used as the estimate of $\mu$.

An example of the calculation of $Q$ and $\chi'$ is given in § 7.

## 6. Methods of estimating the average response

As has been pointed out in the second section, the average response to a treatment over a set of experiments is frequently of considerable importance, even when the response varies from experiment to experiment. The problem of how it may best be estimated from the results of the separate experiments must therefore be considered.

If the experiments are all of equal precision the efficient estimate is clearly the ordinary mean of the apparent responses in each experiment, whether the true responses are the same or vary from experiment to experiment. If, on the other hand, some of the experiments are more precise than others, the ordinary mean, by giving equal weight to both the less and the more accurate results, may appear at first sight to furnish a considerably less precise estimate than might be obtained by more refined statistical processes. As will appear from what follows, however, there are several factors which increase the advantages of the ordinary mean in relation to other possible estimates, so that unless the experiments differ widely in accuracy, or the conditions are somewhat different from those ordinarily met with in agriculture, the ordinary mean is in practice the most satisfactory as well as the most straightforward estimate to adopt.

The simplest alternative to the ordinary mean is the weighted mean mentioned at the end of the last section, in which the weights are inversely proportional to the error variances of the estimates derived from

the various experiments. This weighted mean would be the efficient estimate if there were no variation in the true response from experiment to experiment, and if, moreover, the error variances of the experiments were accurately known. If the error variances are only estimated from a small number of degrees of freedom, however, the weighted mean loses greatly in efficiency and is frequently less efficient than the unweighted mean.

If the true response varies from experiment to experiment, having a variance of $\sigma_0^2$, and the error variances are accurately known, the efficient estimate of the mean response is provided by a weighted mean with weights inversely proportional to $\sigma_0^2+\sigma_1^2, \sigma_0^2+\sigma_2^2 \ldots$, where $\sigma_1^2, \sigma_2^2, \ldots$, are the error variances of the estimates from the various experiments. This has been called the *semi-weighted mean*, since the weights are intermediate between those of the weighted mean and the equal weights of the ordinary mean.

If the response does not vary from experiment to experiment, but the error variances are not accurately known, being estimated from $n_1$, $n_2$, ..., degrees of freedom, the efficient estimate is obtained by the solution of the maximum likelihood equation:

$$\mu = S\,\frac{(n_i+1)\,x_i}{n_i s_i^2+(x_i-\mu)^2}\Big/ S\,\frac{(n_i+1)}{n_i s_i^2+(x_i-\mu)^2}.$$

This solution has the effect of giving lower weights to the more discrepant values than would be given by the ordinary weighted mean. It is not difficult to solve the equation by successive approximation, starting with a value of $\mu$ equal to the unweighted mean, but since in agricultural experiments cases in which the response can confidently be asserted not to vary are rare, the additional numerical work is not ordinarily justifiable, except when exact tests of a significance are required and when the $n_i$ are small.

Thus the available rigorous methods of weighting are not of much use in the reduction of the results of the type ordinarily met with. On the other hand, when a set of experiments of widely varying accuracy is encountered, some method of discounting the results of the less accurate experiments is required. The simplest method would be to reject the results of the less accurate experiments entirely, but this involves the drawing of an arbitrary line of division. Anyone who has attempted this will know how easy it is in certain cases to produce substantial changes in the mean response by the inclusion or exclusion of certain border-line experiments.

An alternative procedure is that of fixing an upper limit to the weight assignable to any one experiment. All experiments having error variances which give apparent weights greater than this upper limit are treated as of equal weight. Experiments having a lesser accuracy are weighted inversely as their error variances. The efficiency of this procedure is discussed in Cochran (1937), where it is shown to be substantially more efficient than the use of the ordinary weighted mean if the numbers of degrees of freedom for error are small. Quite large changes in the choice of the upper limit do not seriously affect the efficiency, and equally will not produce any great changes in the resultant estimate. In most agricultural field experiments the upper limit given by an error variance corresponding to a standard error of 5–7 % per plot would seem appropriate in cases in which there is no evidence of variation in the response from experiment to experiment.

A further alternative procedure which produces much the same effect is provided by the use of the semi-weighted mean, assigning some arbitrary value to $\sigma_0^2$. This procedure has the advantage of being easily adaptable to cases in which there is evidence of variation in response from experiment to experiment. If, for instance, there are eight replicates of each treatment, the value of $\sigma_0^2$ corresponding to 4 % per plot will be $(0{\cdot}04)^2\,(\frac{1}{8}+\frac{1}{8})$, i.e. 0·0004 times the square of the mean yield. This will produce about the same effect as taking a lower limit corresponding to a standard error of 5 % per plot. If in addition the estimated variance of the response from centre to centre is 0·0006 times the square of the mean yield, then we might reasonably take a value of $\sigma_0^2$ corresponding to 0·0010 times the square of the mean yield.

If the error variances of the various experiments are accurately known the error variance of any form of weighted mean is given by

$$\frac{w_1'^2\,\sigma_1^2+w_2'^2\,\sigma_2^2+\dots}{(w_1'+w_2'+\dots)^2},$$

where $w_1'$, $w_2'$, ... represent the weights actually adopted. If $w_1'$, $w_2'$, ... are equal to $1/\sigma_1^2$, $1/\sigma_2^2$, ..., this expression reduces to the expression for the error variance of the fully weighted mean, namely $1/(w_1'+w_2'+\dots)$, and if all the weights are equal the error variance of the unweighted mean of $k$ estimates

$$\frac{1}{k^2}\,(\sigma_1^2+\sigma_2^2+\dots)$$

is obtained.

If, however, the error variances are estimated, and the weights depend on these estimates, the above expression will not be correct. In particular

the estimated error variance of the fully weighted mean in a group of experiments each with $n$ degrees of freedom for error will be $n/(n-4)$ times the expression given above (the variances being replaced by their estimates throughout). The error variance of any semi-weighted mean, or weighted mean with upper limit, will have to be similarly increased. No exact expressions are available, but in general the additional factor must lie between $n/(n-4)$ and unity. In the case of the weighted mean with upper limit to the weights the inclusion of the factor $n/(n-4)$ in the terms which have weights below the upper limit is likely to give a reasonable approximation.

The mean (weighted or unweighted) may be tested for significance by means of the $t$ test, using the estimated standard error. The test is not exact, since the number of degrees of freedom is not properly defined, but if a number somewhat less than the total number of degrees of freedom for error in the whole set of experiments is chosen the test will be quite satisfactory.

There is one further point that must be examined before using any form of mean in which the weights depend on the relative precision of the various experiments. If the precision is associated in any way with the magnitude of the response, such a weighted mean will produce biased estimates and must not be used. Thus, for example, the response to a fertilizer might be greater on poor land, and this land might be more irregular than good land, so that experiments on poor land would give results of lower precision. In such a case any of the above weighted means would lead to an estimate of the average response which would be smaller than it should be.

To see whether association of this type exists the experiments may be divided into two or more classes according to accuracy, and the differences between the mean response in each class examined. Alternatively the regression of the responses on the standard errors of the experiments may be calculated.

## 7. Example of the analysis of a set of experiments of unequal precision

Table IX gives the responses (in yield of roots) to the three standard fertilizers in a set of $3 \times 3 \times 3$ experiments on sugar beet. These experiments were conducted in various beet growing districts in England. The results shown are those of the year 1934 and are reported in full in the Rothamsted Report (1934). It cannot be claimed that the sites were selected at random (practical considerations precluded this course) and

consequently any values obtained for the average responses must be accepted with caution, but the results will serve to illustrate the statistical points involved.

Table IX. *Responses to fertilizers in a series of experiments on sugar beet*

Washed roots (tons per acre)

| Station | Mean yield | Linear response to N | P | K | Standard error | Wt. | Degrees of freedom |
|---|---|---|---|---|---|---|---|
| Allscott | 10·97 | −0·24 | +0·63 | +0·57 | ±0·519 | 3·7 | 15 |
| Bardney | 11·44 | +1·23† | +0·35 | +0·01 | ±0·285 | 12·3 | 22 |
| Brigg | 13·42 | +0·11 | −0·38 | −0·21 | ±0·603 | 2·8 | 22 |
| Bury | 13·83 | +2·08† | −0·05 | −0·22 | ±0·351 | 8·1 | 15 |
| Cantley | 12·90 | +0·20 | +0·32 | +0·14 | ±0·453 | 4·9 | 15 |
| Colwick | 10·12 | +1·05† | +0·87† | −0·07 | ±0·287 | 12·2 | 15 |
| Ely | 12·46 | −1·14 | +0·80 | −0·08 | ±0·886 | 1·3 | 15 |
| Felstead | 11·28 | +3·34† | +0·11 | +0·23 | ±0·356 | 7·9 | 15 |
| Ipswich | 12·45 | +1·64† | +0·57 | +0·34 | ±0·344 | 8·5 | 15 |
| King's Lynn | 19·54 | +0·52 | +0·12 | −0·57 | ±0·481 | 4·3 | 15 |
| Newark | 14·10 | +1·37† | +0·54* | −0·33 | ±0·198 | 25·5 | 15 |
| Oaklands | 12·84 | 0·00 | −0·14 | +0·40 | ±0·622 | 2·6 | 15 |
| Peterborough | 17·99 | −0·14 | +1·02 | −1·34* | ±0·618 | 2·6 | 15 |
| Poppleton | 14·21 | +2·72† | −0·21 | −0·18 | ±0·357 | 7·8 | 22 |
| Wissington | 14·55 | +3·32† | +0·19 | +0·38 | ±0·443 | 5·1 | 15 |
| Mean | 13·47 | +1·07 | +0·32 | −0·06 | ±0·125 | 109·6 | 246 |

* 5 % significance. † 1 % significance.

At Bardney, Brigg and Poppleton there were two complete replications, i.e. fifty-four plots, while at each of the remaining centres there was a single replication, twenty-seven plots, only, the error being estimated from the interactions of the quadratic components of the responses and from the unconfounded second order interactions. At all centres the experiments were arranged in blocks of nine plots.

The size of the plot varied. It is immediately apparent, from inspection, or by application of the process described in § 4, that the experiments are of very varying precision. In general the larger plots, as might be expected, gave the more accurate results, though the gain in precision was not proportional to the increase in area.

(*a*) *The response to nitrogen.*

The response to nitrogen clearly varies significantly from centre to centre, this variation being large in comparison with experimental error. The ordinary analysis of variance of these responses is given in Table X.

The pooled estimate of error is equal to the mean of the squares of the standard errors given in Table IX. The estimate of the standard error of the average response is therefore

$$\sqrt{(0{\cdot}2345/15)} = \pm 0{\cdot}125.$$

Table X. *Analysis of variance of response to nitrogen*

| | Degrees of freedom | Sum of squares | Mean square |
|---|---|---|---|
| Average response | 1 | 17·1949 | 17·1949 |
| Response × centres | 14 | 25·5891 | 1·8278 |
| Pooled estimate of error | | | 0·2345 |

Since the errors are unequal the $t$ distribution will not be exactly followed, the actual 5 % point being subject to slight uncertainty, but in any case intermediate between those given by $t$ for 15 and for 246 degrees of freedom.

The variation of the response from centre to centre has therefore an estimated variance of

$$1{\cdot}8278 - 0{\cdot}2345 = 1{\cdot}5933,$$

excluding variance due to error. This method of estimation is not fully efficient, but may be used in cases such as the present in which the variation is large in comparison with the experimental errors.

It is clear that even were the precision of the experiments known with exactitude, the standard errors being those of Table IX, the semi-weighted mean of § 6, which could then be used, would differ little from the unweighted mean, since the weights would only range from

$$\frac{1}{1{\cdot}5933 + 0{\cdot}0392} \text{ to } \frac{1}{1{\cdot}5933 + 0{\cdot}7850},$$

i.e. from 0·61 to 0·42. The unweighted mean is therefore the only estimate of the average response to nitrogen that need be considered. It may be noted that in this set of experiments there appears to be some association between degree of accuracy and magnitude of response to nitrogen. This is an additional reason for not using any form of weighted mean.

(*b*) *The response to superphosphate.*

The responses to superphosphate are of much smaller magnitude than those to nitrogen. Only two, those of Colwick and Newark, are significant, but eleven out of the fifteen are positive, and consequently there is some evidence for a general response.

The unweighted mean of the responses is +0·32 and the standard error of the quantity is, as before, ±0·125. The unweighted mean is therefore significant.

The weights corresponding to the estimated standard errors are given in Table IX. The sum of the products of these weights and the responses to phosphate, divided by the sum of the weights, gives the weighted mean, +0·365. This differs somewhat from the unweighted mean, and

inspection shows that the difference is largely due to the fact that the two stations which gave significant results received high weights. The weight assigned to Newark is nearly $\frac{1}{4}$ of the total weight. The estimated error of this experiment is 3 % per plot, which would appear to be lower than is likely to be attained in practice. Fixing a lower limit of error at 5 % per plot, which is equivalent to a weight of 10·0 for the experiments of twenty-seven plots and of 20·0 to experiments with fifty-four plots, we obtain the weighted mean with upper limit, 0·323.

Following the rule given in § 6, the estimated standard error of the weighted mean will be given by the square root of

$$\frac{3{\cdot}7\times\frac{15}{11}+12{\cdot}3\times\frac{22}{18}+\ldots}{(3{\cdot}7+12{\cdot}3+\ldots)^2}.$$

This gives the value $\pm 0{\cdot}111$. Similarly the estimated standard error of the weighted mean with upper limit to the weights is given by the square root of

$$\frac{3{\cdot}7\times\frac{15}{11}+12{\cdot}3\times\frac{22}{18}+\ldots+\frac{10^2}{12{\cdot}2}+\ldots}{(3{\cdot}7+12{\cdot}3+\ldots+10+\ldots)^2}.$$

This gives a value of $\pm 0{\cdot}112$. This is presumably somewhat of an over-estimate, as this mean is likely to be somewhat more accurate than the weighted mean.

In order to test whether there is any evidence of variation in the phosphate response from centre to centre the weighted sum of squares of deviations $Q$ may be calculated by the formula given at the end of § 5. For convenience in this calculation it is best to tabulate the products $wx$ of the weights and the responses separately for each centre. The values obtained are

$$Swx^2 = 27{\cdot}6068$$
$$\bar{x}_w\, Swx = 14{\cdot}6044$$
$$Q = 13{\cdot}0024$$

The value of $\chi'^2$ may now be calculated. In the present case the number of degrees of freedom for error varies from experiment to experiment. We will take $n$ to be equal to the mean 16·4 of these numbers. This procedure will be satisfactory if the numbers do not differ too widely and are reasonably large in all experiments. Using this value we have

$$\chi'^2 = 14+\sqrt{\frac{12{\cdot}4}{15{\cdot}4}}\left(\frac{14{\cdot}4}{16{\cdot}4}\,13{\cdot}0024-14\right)=11{\cdot}7.$$

Clearly there is no evidence of any variation in response from centre to centre.

It may, however, be considered that although there is no evidence of variation in response, such variation should not be precluded, and that consequently the upper limit of the weights should be lower than the values of 10 and 20 taken above. Fixing the limit at 7·8, so as to give seven of the fifteen experiments equal weight, we obtain a mean of $+0{\cdot}301$, with an estimated standard error of $\pm 0{\cdot}109$. In fertilizer experiments such as the present, where from the nature of the treatments, constancy of response would seem unlikely, this last estimate of the mean response appears to be the most satisfactory, since it gives equal weight to all the more accurate experiments and at the same time prevents the less accurate experiments from unduly influencing the results.

(c) *The response to potash.*

The effect of potash shows no significance, either in mean response or in variation in response from centre to centre. The significant depression at Peterborough can consequently be reasonably attributed to chance.

The analysis follows the lines already given and need not be set out in detail here. The weighted mean with upper limit 7·8 has the value $-0{\cdot}03 \pm 0{\cdot}109$. The value of $\chi'^2$ for testing the significance of the variation in response is 11·6.

The results discussed here are, of course, only a part of the full results of the experiments. No consideration has been given to the curvature of the response curves, or to the interactions of the different fertilizers. The whole set of experiments provides an excellent illustration of the power of factorial design to provide accurate and comprehensive information. It will be noted, among other things, that the mean responses to the three fertilizers are determined with a standard error of less than 1 % of the mean yield.

## SUMMARY

When a set of experiments involving the same or similar treatments is carried out at a number of places, or in a number of years, the results usually require comprehensive examination and summary. In general, each set of results must be considered on its merits, and it is not possible to lay down rules of procedure that will be applicable in all cases, but there are certain preliminary steps in the analysis which can be dealt with in general terms. These are discussed in the present paper and illustrated by actual examples. It is pointed out that the ordinary analysis of variance procedure suitable for dealing with the results of a

single experiment may require modification, owing to lack of equality in the errors of the different experiments, and owing to non-homogeneity of the components of the interaction of treatments with places and times.

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# NUTRITIVE VALUE OF PASTURE

## XII. THE INFLUENCE OF CUTTING AT MONTHLY INTERVALS OVER NINE SEASONS ON THE QUALITY AND PRODUCTIVITY OF A HEAVY-LAND PASTURE

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### Introduction

When this investigation was begun in 1929, the conception of intensive grassland management, involving rotational grazing and the liberal use of manures, including artificial nitrogenous fertilizers, was just coming into prominence in this country. It had become necessary to secure information about the improvement of yield and composition of herbage that could be brought about by such a system of management, since somewhat extravagant claims were being made for the magnitude of the effect of sulphate of ammonia and other soluble nitrogenous manures on the yield and composition of pastures.

It was decided, therefore, to begin an investigation into the influence of intensive nitrogenous fertilizing on the yield and composition of a pasture, the soil of which contained adequate reserves of lime, potash and phosphate. A report covering the first two years of this work (1929 and 1930) was published some years ago (Woodman & Underwood, 1932). It was shown that large pasture plots receiving ground limestone, superphosphate, sulphate of potash and farmyard manure averaged, under a system of monthly cuts, 90 % of the dry-matter yield of similar plots receiving the same manures and, in addition, periodic small dressings of sulphate of ammonia throughout the growing season. This result suggested that the improvement of yield from the use of soluble nitrogen might in certain circumstances be not so considerable as had been claimed by the advocates of this system of fertilizing. The paper further dealt with (1) the influence of different systems of fertilizing on the *seasonal* productivity of the pasture and on the chemical and botanical composition of the herbage; (2) the percentage recovery in the herbage of the applied nitrogen, potash and phosphoric acid.

Since the publication of this first paper, it has been demonstrated in

numerous similar investigations that the magnitude of the yield improvement from intensive fertilizing depends primarily on the initial state of fertility of the pasture, and that although the most striking effects may be experienced on grassland in a low state of fertility (e.g. natural pastures, where the manurial reserves in the soil may have suffered far-reaching depletion), the improvement may be quite inconsiderable when the same manures are used on cultivated pasture "in good heart".

Indeed, interest in this aspect of the problem began to wane as successive investigations served to clarify the position. Meanwhile, however, another question had forced itself to the fore, consequent on the practical interest that was beginning to be manifested in the proposal for cutting young, leafy grass at intervals throughout the season for conservation either by artificial drying or by ensilage (Woodman *et al.* 1927). The question arose as to how pastures would stand up to the form of cutting involved in this system of conservation. Would there be a gradual decline in the quality and productivity of grassland submitted to such treatment? One of the main objects of continuing this work over a period of 9 years (1929–37, inclusive) has been to secure information which might enable this question to be answered.

### The lay-out of the pasture plots

The reader is referred to the earlier publication for a detailed account of the general arrangement of the trials (Woodman & Underwood, 1932) which were carried out on a pasture situated on the heavy land of the University Farm, Cambridge. An oblong plot measuring 361 by 200 links was divided into thirteen equal subplots, each comprising $\frac{1}{20}$ acre ($25 \times 200$ links) and separated from adjacent subplots by three-link borders. The arrangement is shown in Diagram I, and the complete details of manurial treatment throughout the 9 years of the experiment are shown in Table I.

| A1 | B2 | A3 | C4 | A5 | B6 | C7 | A8 | B9 | A10 | C11 | A12 | D13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Section I | | | | Section II | | | | Section III | | | | |

Diagram I. Showing lay-out and treatment of subplots.

A subplots: CaO, $P_2O_5$ and $K_2O$ with periodic applications of N.
B subplots: CaO only.
C subplots: CaO, $P_2O_5$ and $K_2O$.
D subplot: No applications of CaO or artificial fertilizers.
All subplots: Autumnal applications of farmyard manure.

It should be added that the subplots were cut at monthly intervals, eight such cuts being obtained during each season. The subplots in

section II (A5 to A8) were always cut a week later than those in section I (A1 to C4) and those in section III (B9 to D13) a week after those in section II. A fortnight was then allowed to pass before cutting the subplots in section I again, so that in this way the field work was spread out and every subplot was permitted four weeks of unchecked growth between successive cuttings.

Table I. *Complete details of manurial treatment*

| | |
|---|---|
| *Autumn*, 1926. | 10 cwt. basic slag per acre to whole pasture. |
| *January*, 1929. | A and C subplots: Ground limestone (2 tons per acre).<br>"Super" (5 cwt. per acre).<br>Sulphate of potash (2 cwt. per acre).<br>A subplots also received seven dressings (each $\frac{5}{8}$ cwt. per acre) of sulphate of ammonia at intervals during season.<br>B subplots: Ground limestone (2 tons per acre). |
| *Late autumn*, 1929. | 5 tons per acre of F.Y.M. to all subplots. |
| *Season* 1930. | A subplots received six dressings (each $\frac{5}{8}$ cwt. per acre) of sulphate of ammonia.<br>All subplots received F.Y.M. (5 tons per acre) in late autumn. |
| *Season* 1931. | A subplots received six dressings (each $\frac{5}{8}$ cwt. per acre) of sulphate of ammonia. |
| *Late autumn*, 1931. | All subplots received F.Y.M. (5 tons per acre).<br>A and C subplots: "Super" (5 cwt. per acre).<br>Sulphate of potash (5 cwt. per acre). |
| *Season* 1932. | A1, A5 and A10: $3\frac{1}{8}$ cwt. per acre of sulphate of ammonia in one dressing during February.<br>A3, A8 and A12: Five dressings of sulphate of ammonia (each $\frac{5}{8}$ cwt. per acre) in February, March, April, May and July.<br>All subplots received F.Y.M. (5 tons per acre) in late autumn. |
| *Season* 1933. | A1, A5 and A10: Five dressings of sulphate of ammonia (each $\frac{5}{8}$ cwt. per acre) in February, March, April, May and July.<br>A3, A8 and A12: $3\frac{1}{8}$ cwt. per acre of sulphate of ammonia in one dressing during February.<br>All subplots received F.Y.M. (5 tons per acre) in late autumn. |
| *Season* 1934. | No artificials to any of subplots.<br>F.Y.M. (5 tons per acre) to all subplots during late autumn. |
| *Season* 1935. | No artificials to any of subplots during cutting season.<br>F.Y.M. (5 tons per acre) to all subplots during late autumn.<br>A and C subplots: "Super" and sulphate of potash (each 5 cwt. per acre) during late autumn. |
| *Season* 1936. | A3, A5 and A12: Five dressings of sulphate of ammonia (each $\frac{5}{8}$ cwt. per acre) in February, March, April, July and August. |
| *Late autumn*, 1936. | All subplots save D13: "Super" and sulphate of potash, each at 5 cwt. per acre.<br>A3, C4, B9 and A12: F.Y.M. ($5\frac{1}{2}$ tons per acre).<br>B2, A5, A8 and C11: Dried poultry manure ($1\frac{1}{2}$ tons per acre).<br>A1, B6, C7, A10 and D13: No organic manure. |
| *Season* 1937. | All subplots save D13: 1 cwt. per acre of sulphate of ammonia in April and at rate of $\frac{5}{8}$ cwt. per acre in July. |

## INFLUENCE OF REPEATED MONTHLY CUTTING ON PRODUCTIVITY

It is not feasible, from considerations of space, to record the yields of individual subplots at every monthly cutting throughout the 9 years of experiment, but since the differences observed between the average total

yields of the A, B and C subplots were reflected with a high degree of consistency in the comparisons between the individual A, B and C subplots in each of the three sections, the former figures only need be given for the purposes of this discussion (see Table II). The monthly variations of yield during any particular season will be dealt with later (see Table III).

Table II. *Average total yields, in terms of lb. dry matter, from A, B, C and D subplots in the different years of experiment*

| Season | A lb. D.M. per acre | C lb. D.M. per acre | B lb. D.M. per acre | D lb. D.M. per acre | Character of season | Rainfall from April to Sept. (incl.) in. |
|---|---|---|---|---|---|---|
| 1929 | 3982 | 3626 | 3266 | 2956 | Generally droughty, particularly in Mar., June, Aug. and Sept. | 7·58 |
| 1930 | 7996 | 7102 | 6824 | 6126 | Abundant and well-distributed rainfall | 14·59 |
| 1931 | 8274 | 7334 | 7248 | 7055 | Abundant and well-distributed rainfall | 17·21 |
| 1932 | 6402 | 5634 | 5596 | 5542 | June hot and dry | 13·35 |
| 1933 | 5149 | 5006 | 4568 | 4470 | Favourable conditions in Mar. Severe drought throughout summer | 9·39 |
| 1934 | 3000* | 3477 | 3398 | 2861 | Dry cold spring; droughty until end of July | 9·42 |
| 1935 | 4022* | 4565 | 4500 | 4029 | Favourable Mar.; good total rainfall, but cold and dry in May; droughty in July and first half of Aug. | 13·19 |
| 1936 | 6439† | 5584 | 5791 | 5577 | Good total rainfall, but May cold and dry; Aug. also dry | 13·50 |
| 1937 | 7800‡ | 7700 | 7730 | 7066 | Favourable weather throughout | 13·35 |

* No nitrogen applied to A subplots in these years.

† *Average for A subplots receiving nitrogen in this year:* A 3, A 5 *and* A 12 *(see* Woodman & Evans, 1938).

‡ *All subplots save* D 13 *received nitrogen in this year.*

### *Comments on Table II*

The results in Table II emphasize the predominant influence of rainfall on the yield of pastures. In the first season of the work (1929), the cold, dry spring and the drought of summer are reflected in the low yields from all the subplots, irrespective of manurial treatment. Then followed two seasons of exceedingly favourable conditions for growth of grass, and the yields rose to very high levels. Indeed, during 1931, the A subplots averaged a yield of herbage equivalent, on the basis of a 10% moisture content, to about 4·1 tons of dried young grass per acre.

The yields declined somewhat in the following season (1932), mainly as a consequence of poorer growth during the second half of the season as

compared with the corresponding period of 1931. The decline became even more marked in 1933, the summer of which was one of the hottest and droughtiest on record. Heat-wave and drought persisted right into September and the pasture assumed a brown, bare appearance. No cuts were taken from any of the subplots during the September of this year.

This year was unfortunately followed by another droughty season in 1934. Unfavourable conditions for growth of grass were again experienced, and this circumstance, superimposed on the harmful effects of the previous season's drought, caused the productivity of the pasture to fall to the lowest level manifested throughout the whole 9 years of the trial. No cuts were possible during the July of this year, and the appearance of the subplots suggested that two successive years of drought could do more to endanger quality and productivity in pastures than neglect of any of the controllable factors of management.

The season of 1935 saw a return to more normal weather conditions, excepting that May, which should have heralded the flush, was this year exceptionally cold and dry. There was a remarkably severe frost as late as 17 May, and the growth of grass at this early stage of the season was disappointing. The subplots suffered also from dry conditions in July. Nevertheless, even though no nitrogen was applied to any of the subplots in this season, the recovery in productivity was very satisfactory when account is taken of the fact that the disadvantageous influence of the previous two seasons of drought could still be traced in the appearance of the subplots. The improvement was particularly noticeable in subplot D13, which had received no aid from artificial fertilizers throughout the whole of the years of experiment.

The total rainfall in the 1936 season was ample, but a cold, dry May again affected the "flush" growth adversely. Very abundant rainfall, however, was registered in June and July, and at this time the subplots presented a splendid appearance, the harmful effects of the previous years of drought seeming at length to have been overcome. The pasture during this season yielded as well as it did in 1932, the year immediately preceding the two droughty seasons.

The final season (1937) was in every way a favourable year for growth of grass. It will be noted that the subplots gave very heavy yields, which compared well with those obtained in the excellent seasons of 1930 and 1931 (seasons 2 and 3). Indeed, the B and C subplots gave their heaviest yields for the whole of the trial, but this superiority over the 1931 season was no doubt due partly to a small dressing of sulphate of ammonia applied to these subplots in 1937 (see Table II). The A subplots averaged

a yield of herbage equivalent to 3·86 tons of dried young grass (containing 10 % of moisture) per acre.

The case of subplot D 13 is of special interest. This subplot received, throughout the trials, neither lime nor artificial fertilizers, but merely the moderate autumnal dressings of farmyard manure. Even this was omitted prior to the 1937 trial; yet during this final season, D13 produced a heavier yield of herbage than in any previous year. It may be, however, that such a result could only be obtained on soils similar to that of the experimental pasture, namely, a heavy soil initially "in good heart" and well supplied with lime.

It is readily apparent from Table II, therefore, that after nine seasons of continuous monthly cutting, including two successive seasons in which severe heat and drought had exerted a most adverse effect on the vigour of the herbage, none of the subplots had displayed any signs of deterioration in productivity.

## Influence of repeated monthly cutting on quality of herbage

### (1) *Quality as based on chemical composition of herbage*

The crude protein content of young grass is now generally recognized as affording a fairly reliable criterion of feeding value. It may be assumed *that if the dry matter of the grass contains 17 % or more of crude protein,* the product that would be obtained by the artificial drying of such herbage will have a digestibility and starch equivalent comparable with those of concentrated foods.

It would need too much space to give the percentages of crude protein in all the cuts from the individual subplots throughout the 9 years of the investigation, and it must suffice to record the averages of the values for the thirteen subplots at each monthly cutting. The values are summarized in Table III, and the average yields of the subplots at every monthly cut are also given. From this table, therefore, information may be derived in respect of the seasonal variation of the yields both of dry matter and crude protein. That the curves of seasonal productivity in the various years of experiment would show considerable differences is at once apparent, and the significance of the figures can only be understood fully by considering them in relation to the character of the weather at the different periods of each season (see Table II).

Table III. *Seasonal variations of yield and crude protein content (dry matter basis) in the different years of experiment (average values for all subplots)*

| Year | | Late Mar.–Apr. | Apr.–May | May–June | June–July | July | Aug. | Sept. | Oct. |
|---|---|---|---|---|---|---|---|---|---|
| 1929 | lb. D.M. per acre | — | 653 | 1476 | 720 | 343 | 264 | 61 | 138 |
| | % C.P. | — | 25·5 | 20·8 | 19·9 | 21·3 | 21·1 | 20·4 | 23·7 |
| 1930 | lb. D.M. ,, | 1103 | 1303 | 1683 | 933 | 561 | 937 | 479 | 376 |
| | % C.P. | 24·6 | 22·3 | 18·5 | 20·0 | 20·3 | 20·7 | 21·4 | 23·3 |
| 1931 | lb. D.M. ,, | 858 | 1010 | 1626 | 1055 | 626 | 936 | 854 | 762 |
| | % C.P. | 23·3 | 20·9 | 16·1 | 17·0 | 18·7 | 19·0 | 20·0 | 23·3 |
| 1932 | lb. D.M. ,, | 1036 | 673 | 1522 | 673 | 582 | 571 | 568 | 347 |
| | % C.P. | 24·4 | 22·5 | 16·6 | 17·6 | 18·7 | 19·1 | 20·9 | 21·8 |
| 1933 | lb. D.M. ,, | 1141 | 891 | 950 | 627 | 594 | 258 | — | 469 |
| | % C.P. | 20·1 | 20·3 | 18·0 | 20·5 | 20·8 | 20·2 | — | 24·5 |
| 1934 | lb. D.M. ,, | 421 | 801 | 854 | 286 | — | 241 | 182 | 407 |
| | % C.P. | 23·4 | 22·4 | 19·3 | 19·5 | — | 19·1 | 21·5 | 22·6 |
| 1935 | lb. D.M. ,, | 1121 | 802 | 582 | 628 | 239 | 103 | 280 | 505 |
| | % C.P. | 19·4 | 19·1 | 17·7 | 17·3 | 16·6 | — | 22·3 | 21·6 |
| 1936 | lb. D.M. ,, | 765 | 579 | 942 | 590 | 763 | 1076 | 670 | 448 |
| | % C.P. | 22·6 | 20·7 | 16·0 | 18·6 | 17·8 | 20·9 | 24·1 | 22·4 |
| 1937 | lb. D.M. ,, | 1185 | 1440 | 1234 | 1119 | 1164 | 694 | 414 | 454 |
| | % C.P. | 18·3 | 19·7 | 18·1 | 22·2 | 24·2 | 25·6 | 27·4 | 25·8 |

*Note.* For comments on weather conditions, see Table II.

### *Comments on Table III.*

It will be noted that only on four occasions did the percentage of crude protein, on the basis of dry matter, fall below the critical value of 17 %. Two of these cases were encountered during the May–June period of 1931 and 1932, when heavy flush growths were obtained. In order to have kept the crude protein content at more than 17 %, it would merely have been necessary at this stage to have shortened the interval between successive cuts from 4 to 3 weeks. The third case occurred during the corresponding period of 1936. In this case, however, the flush growth was disappointingly thin and light in consequence of the abnormally cold and dry May of this year, and the relatively low crude protein content at this stage was merely a reflection of the poor quality of the herbage produced under these inclement conditions. The fourth case (July 1935) was also attributable to the effect of adverse weather conditions, the subplots at this stage presenting a bare and "scorched" appearance as a result of prolonged heat and drought.

If the influence of the varying weather conditions in the different years be taken into account, it is possible to conclude that the continuous monthly cutting over the nine seasons of experiment had caused no falling off in the quality of the pasturage from the standpoint of chemical composition as measured by crude protein content. The extremely high values obtained in the second half of the final season (1937), when

the herbage contained a very good proportion of wild white clover, will particularly be noted.

This conclusion is further substantiated if the average yield of crude protein from the subplots in 1937 is compared with the corresponding results for the extremely favourable seasons of 1930 and 1931. The figures, in terms of lb. crude protein per acre, are 1662 lb. (1937), 1558 lb. (1930) and 1495 lb. (1931). In comparing these figures, it should be remembered that, in 1937, all the subplots save D 13 received sulphate of ammonia, whereas in the earlier years only the A subplots were dressed with this fertilizer.

### (2) *Quality as based on botanical composition of herbage*

Under the influence of a season of well-distributed rainfall, the subplots during the final year (1937) presented a splendid appearance, the herbage continuing to grow strongly and vigorously throughout the whole period from April to mid-October. It was the verdict of competent observers that the pasture had never shown to better advantage at any prior stage of the trials. There were certainly no signs during this final year that the system of monthly cuts adopted throughout the 9 years had caused any falling off in the quality of the herbage as judged by eye. The prompt recovery of the subplots after every monthly cutting gave evidence of the ability of the herbage plants to respond to the favourable weather conditions of this season. The growth on all the subplots was of a grassy character, with a thick "bottom" growth and a very desirable blending of wild white clover. Weeds were scarce and contributed but little to the mown herbage.

The influence of continuous monthly cutting on the quality of the pasturage has been investigated under what must be admitted to have been exceedingly severe conditions in view of the two successive summers of almost exceptional heat and drought (1933 and 1934) that came within the nine years over which the experiment lasted. Indeed, it could not but be concluded that two such years, coming in succession, are liable to damage pasture quality in a greater degree than the neglect of any of the controllable factors constituting good management. If the system of cutting could have damaged the pasture in a permanent sense, this would surely have happened during these droughty years. It was astonishing how weeds, dandelions in particular, established themselves in the sward as a result of the lack of effective competition from the grasses. That the recovery which was manifested during the subsequent seasons of monthly cutting (1935 and 1936) was of a substantial

character is shown by the remarkably good results of the 1937 season, and in itself is evidence of an absence, under the conditions of the experiment, of ill-effects directly attributable to the system of cutting.

The treatment of the subplots appeared to bring about no considerable modification in the distribution of the species of grasses. The most abundant species during the first season of the trials were rough-stalked meadow grass, perennial rye grass, creeping bent and red fescue; whilst smaller amounts of cocksfoot, meadow foxtail, crested dogstail and timothy were also present. All these species could be found in the subplots during the final season, and the grasses that had been abundant at the beginning were still represented plentifully at the end. Cocksfoot, owing to its quicker recovery after the droughty years, had become somewhat more prominent, whilst barley grass had persisted strongly in the furrows of the subplots.

The most outstanding effect was that produced by the sulphate of ammonia applied to the A subplots in leading to a marked reduction of such weeds as daisies and buttercups. The regular cutting did not unduly encourage the growth of wild white clover at the expense of the grasses. The relatively heavy dressings of sulphate of ammonia applied to the A subplots from 1929 to 1933 certainly tended to restrict somewhat the growth of the clover on these subplots as compared with the B and C subplots, but during 1934 and 1935, when no artificial nitrogen was applied, wild white clover, under the influence of the regular cutting, underwent a marked stimulation in the A subplots. During the final season, there was little to distinguish the different subplots in this respect.

### THE USE OF DRIED POULTRY MANURE ON PASTURES

It will already have been noted that during 1937 the subplots were subjected to a very different manurial treatment from that of previous seasons. It had been decided this year to begin an investigation into a new aspect of the problem; namely, whether, under the system of cutting associated with the conservation of young grass either by ensilage or artificial drying, it is possible to maintain quality and productivity by the use of artificial fertilizers only, or whether the use of humus-providing organic manures, such as farmyard manure, is indispensable.

It is recognized that certain advocates of grass drying, more especially commercial concerns working on a factory scale, are tending to dissociate the process from actual farming systems. In consequence, the grassland submitted to regular cutting may receive no organic manure either from being grazed at suitable times or from autumnal dressings of farmyard

manure; artificial fertilizers, in particular sulphate of ammonia, are being relied on for upkeep of fertility. Such a procedure may conceivably have very undesirable consequences.

In such cases the transport from a distance of farmyard manure for autumnal application may not be feasible. Dried poultry manure, on the other hand, would lend itself to such transport, although in present circumstances the supply is too small, and the price too high, for it to be used in this way. Nevertheless, it appeared desirable to find out whether dried poultry manure could be used on pastures as an effective substitute for farmyard manure.

With a view to throwing light on these questions, the twelve subplots from A1 to A12 were all given a dressing of superphosphate of lime and sulphate of potash, each at the rate of 5 cwt. per acre, in the autumn of 1936. Four of the subplots were also given farmyard manure (5–6 tons per acre); another four received an equal dressing, on the basis of dry weight, of dried poultry manure ($1\frac{1}{2}$ tons per acre), whilst the remaining four subplots were given no organic manure. All the subplots received sulphate of ammonia (1 cwt. per acre) in the April of 1937 and again at the rate of $\frac{5}{8}$ cwt. per acre during July. The lay-out of the subplots and the yield results under the system of monthly cuts are shown in Table IV.

Table IV. *Treatment and yields* (*lb. dry matter per* $\frac{1}{20}$ *acre*) *during* 1937

| Section I | | | Section II | | | Section III | | | Section IV | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | B2 | A3 | C4 | A5 | B6 | C7 | A8 | B9 | A10 | C11 | A12 |
| No O.M. | P.M. | F.Y.M. | F.Y.M. | P.M. | No O.M. | No O.M. | P.M. | F.Y.M. | No O.M. | P.M. | F.Y.M. |
| 399·1 | 383·3 | 419·6 | 377·5 | 381·2 | 396·1 | 380·1 | 399·3 | 380·2 | 398·2 | 397·4 | 342·6 |

| | lb. D.M. per acre |
|---|---|
| *Average of subplots receiving no* O.M. | 7868 |
| Average of subplots receiving dried P.M. | 7806 |
| Average of subplots receiving F.Y.M. | 7600 |

Unfortunately, owing to pressure of other work, the investigation had to be abandoned after only one season, so that the results are given without comment. It would obviously be necessary to continue such an experiment over a number of seasons before the effect of omitting organic manure from the scheme of fertilizing would be clearly discernible. The influence of an autumnal dressing of organic manure in stimulating early growth in the following spring deserves mention, however. In the first cut during April, the subplots receiving farmyard manure averaged 1225 lb. dry matter per acre, those receiving dried poultry manure 1333 lb., whilst those which had been given no organic manure averaged only 965 lb. In the case of farmyard manure the result might have been

attributed to the manner in which the dung protects the roots of the grasses from the frosts during winter, but this explanation would not account for the effect of the dried poultry manure, which was completely washed into the soil very soon after its application. In all probability, therefore, the stimulation of early growth manifested by both the farmyard manure and the dried poultry manure must be ascribed to a direct effect of their manurial constituents.

## CONCLUSIONS

A permanent pasture on heavy clay soil has been submitted to cutting at monthly intervals over a period of nine seasons. During this period, varying weather conditions have been experienced, from two consecutive seasons of heat and drought to seasons of abundant and well-distributed rainfall. No deterioration of productivity, ascribable to the system of cutting, has been noted on a number of plots on the pasture which have been submitted to different manurial treatments; nor has the continuous cutting led to any falling off in the quality of the herbage as judged from the standpoints of both chemical and botanical composition.

The question as to whether the fertility and productivity of grassland, under the system of cutting demanded by the modern process of conserving young grass by artificial-drying or by ensilage, can be maintained by artificial fertilizers alone, without the help of humus-providing organic manures such as farmyard manure, is not answered by the results of the present investigation. All the plots in the present trial received autumnal dressings of farmyard manure.

The results from a single investigation, even though the latter is continued over a period of years, are necessarily too restricted in significance to enable generalizations to be made. It is still held, therefore, that the only adequate insurance against the risk of deterioration of fertility and productivity of grassland utilized for the production of young, leafy grass for purposes of conservation is the adoption of the dual technique of cutting and grazing; that is to say, cutting the pasture one year and grazing it the next, or, alternatively, taking but one or two cuts during any one year and arranging for the field to be grazed at other convenient times of the season.

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# NUTRITIVE VALUE OF PASTURE

## XIII. AN INQUIRY INTO THE RESIDUAL EFFECTS OF THE INTENSIVE USE OF SULPHATE OF AMMONIA ON PASTURES

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### Introduction

This publication should be read in conjunction with the paper immediately preceding it in the present issue of this *Journal* (Woodman & Evans, 1938). It deals with a particular phase of the general problem described in the first paper, to which reference should be made for details about the wider objects of the trials, the design of the experiments and the different manurial treatments to which the subplots were subjected.

In a paper published some years ago, dealing with the results of the first two years of this investigation, Woodman & Underwood (1932) showed that of the 189 lb. of nitrogen applied per acre to the A subplots during 1929 and 1930 in the form of periodic dressings of sulphate of ammonia, only 49·6 lb., or 26 %, was accounted for by the nitrogen in the extra herbage produced as a result of applying the nitrogenous fertilizer. This did not signify, however, that the remaining 139·4 lb. of nitrogen from the sulphate of ammonia represented a storage of nitrogen in the soil, as will be clear from the figures in Table I.

Table I. *Total yields of nitrogen from A and C subplots during* 1929 *and* 1930 *compared with amount of nitrogen applied to subplots*

| | A subplots* lb. per acre | C subplots lb. per acre |
|---|---|---|
| Nitrogen removed in herbage (1929 + 1930) | 413·3 | 363·7 |
| Nitrogen applied as sulphate of ammonia | 189·0 | — |
| Nitrogen applied as farmyard manure | 134·0 | 134·0 |
| *Total nitrogen applied* | 323·0 | 134·0 |

* A and C subplots received same treatment in respect of dressings of limestone, "super" and sulphate of potash.

From Table I it would appear that the A subplots, despite the use of sulphate of ammonia, were being depleted of nitrogen, under the conditions of the experiment, at the rate of about 45 lb. per acre per season. This, however, must be regarded as a minimum figure, since it rests on the

dubious assumptions that the whole of the nitrogen of the farmyard manure was incorporated into the soil of the pasture and that none of the nitrogen from the dressings was washed out of the soil. Against this, on the other hand, must be set the fact that the wild white clover in the subplots was able to utilize atmospheric nitrogen. The C subplots, to which no sulphate of ammonia was applied, appeared to be suffering nitrogen depletion at the rate of about 115 lb. per acre per season, but since the growth of wild white clover in these subplots was, during 1929 and 1930, more profuse than in the A subplots, it may be assumed that the conserving effect of this factor on the soil reserves of nitrogen was more in evidence on the C than on the A subplots.

From the foregoing considerations, therefore, it could not be anticipated that the generous applications of sulphate of ammonia to the A subplots would lead to an accumulation of extra reserves of nitrogen in the soil, although only 26 % of the applied nitrogen was being recovered in the extra herbage produced from such dressings. The nitrogen of the sulphate of ammonia was serving, to a large extent, to reduce the rate at which the soil, under the conditions of this experiment, would otherwise have been depleted of its nitrogenous reserves.

## Present investigation

After five seasons of continuous cutting of the subplots at monthly intervals (1929–33, inclusive), during which time the A subplots had received, in all, 18¼ cwt. per acre of sulphate of ammonia, it was decided to submit this question of residual effect to practical test. During the

Table II. *Average yields of subplots in lb. dry matter per acre*

| | A lb. D.M. per acre | | C lb. D.M. per acre | B lb. D.M. per acre | D lb. D.M. per acre |
|---|---|---|---|---|---|
| 1929–1933 (incl.)* | 31,803‡ | | 28,702 | 27,502 | 26,149 |
| 1934† | 3,000 | | 3,477 | 3,398 | 2,861 |
| 1935† | 4,022 | | 4,565 | 4,500 | 4,029 |
| | (*) A3, A5, A12 | (†) A1, A8, A10 | | | |
| 1936 | 6,439 | 5,601 | 5,584 | 5,791 | 5,577 |

A subplots: CaO, $P_2O_5$ and $K_2O$ with periodic applications of N.
C subplots: CaO, $P_2O_5$ and $K_2O$.
B subplots: CaO only.
D subplot: Neither CaO nor artificial fertilizers.
All subplots: Autumnal dressings of F.Y.M.

* Sulphate of ammonia applied to A subplots.
† No sulphate of ammonia applied to A subplots.
‡ See Woodman & Evans, 1938.

next two seasons (1934 and 1935) none of the subplots received sulphate of ammonia, although the autumnal dressings of farmyard manure (5 tons per acre) were applied as before. The yields of the subplots were determined under a system of monthly cuts. During 1936, three of the A subplots (A 3, A 5 and A 12) were again given sulphate of ammonia (five dressings, each at the rate of ⅝ cwt. per acre, during February, March, April, July and August) while the remaining A subplots (A 1, A 8 and A 10) received none.

The yield results, together with other information relevant to the question, are given in Table II.

*Comments on Table II*

The outstanding feature of the results is the temporary severe setback to productivity experienced by the A subplots when the use of sulphate of ammonia was discontinued in 1934. Over the period 1929–33, when this fertilizer was being applied to the A subplots, the latter produced almost 11 % more dry matter than the comparable C subplots. During 1934, however, the position was reversed, the C subplots producing 15·9 % more dry matter than the A subplots. The effect was again clearly observable in 1935, the C subplots again out-yielding the A subplots to the extent of 13·5 %. The average yield of the A subplots in this year was almost equal to that of subplot D 13, the manurial treatment of which had been restricted throughout the trials to the dressings of farmyard manure applied every autumn to all the subplots.

That this effect of discontinuing the use of sulphate of ammonia on the A subplots was manifested with a high degree of consistency is shown by the results in Table III for the individual subplots. It may be noted that the effect was most marked during the flush period of growth (late May—early June). Side by side with this marked fall of productivity of the A subplots in the 1934 season was noted a parallel effect on the protein content of the herbage. During this season the percentage of crude protein (dry matter basis) in the monthly cuts from the A subplots varied from 17·4 to 22·9 %, with a mean value of 19·9 %. The range of variation for the herbage of the C subplots was from 20·1 to 24·6 %, with a mean value of 22·6 %. This effect on composition, however, appears to have been less persistent than the effect on yield, since it was not observable during the following season of 1935, a result probably to be ascribed to the fact that differences in clover content between the A and C subplots had largely been eliminated by the summer of 1935.

Table III. *Yields, in lb. dry matter per $\frac{1}{20}$ acre, of the individual subplots during* 1934 *and* 1935

| | A1 | B2 | A3 | C4 | A5 | B6 | C7 | A8 | B9 | A10 | C11 | A12 | D13 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1934 | 154·8 | 164·0 | 152·9 | 187·5 | 154·7 | 174·9 | 176·6 | 157·5 | 170·7 | 146·5 | 157·3 | 133·6 | 143·1 |
| 1935 | 230·8 | 233·6 | 205·6 | 243·6 | 200·4 | 232·3 | 232·7 | 198·4 | 209·2 | 191·5 | 208·5 | 180·0 | 201·4 |
| | Section I | | | | Section II | | | | Section III | | | | |

## Discussion

It is not easy to account with certainty for the striking manner in which the yields of the A subplots fell behind those of the C subplots following the discontinuance of the use of sulphate of ammonia. The somewhat more luxuriant growth of wild white clover on the C subplots and the moisture-conserving effect of this plant during dry periods may have accounted for some part of the effect, but with the cessation of the sulphate of ammonia applications, the clover began to display stronger growth on the A subplots and the difference in this regard between these subplots and the B and C subplots became much less marked than in the previous years.

During the May of both 1934 and 1935, the thinner growth of herbage on the A subplots was very apparent to the eye. In particular, the B and C subplots had a thicker growth of "bottom" grass, and it seemed possible that the more luxuriant growth on the A subplots in the previous years might have weakened the "bottom" growth in the same way as the growth of clover was depressed, namely, by the smothering effect of the taller grasses. This would further explain why the effect was most marked during the flush period. Whatever the explanation, however, there could be little doubt that this advantage in respect of density of "bottom" growth was in the main responsible for the superior productivity of the B and C subplots during 1934 and 1935.

That any part of the effect could be attributed to the removal of bases from the soil by the repeated applications of sulphate of ammonia in the previous years was ruled out, since the soil of the A subplots as well as that of the B and C subplots still showed a faintly alkaline reaction to bromthymol blue at the completion of the experiments. It should also be kept in mind that the humus supply of the soil was maintained by the autumnal dressings of farmyard manure.

It was also possible that the continuous stimulating of growth by means of sulphate of ammonia, combined with the effect of the regular monthly defoliation of the grasses, might have exerted an adverse

influence on the maintenance of the organic reserves in the roots of the grasses in the A subplots, and that this impoverishment of the root reserves may have manifested itself in a diminished vigour of growth when, during 1934 and 1935, the stimulating influence of sulphate of ammonia was missing.

It is difficult to submit hypotheses such as the foregoing to experimental verification on large subplots containing heterogeneous growths of herbage. A large number of turves (18 in. square) were taken up at various points on the different subplots during the autumn of 1934. There appeared to be little difference among the various subplots in the depths to which the roots penetrated the soil. Most of the roots occupied the top 2–3 in., those reaching down as far as 6 in. forming a small proportion in all cases. If differences did exist between the differently treated subplots in respect either of amonnt of root tissne or depth of penetration, they were certainly not marked enough to be obvious to the eye. Clearly, however, stimulation of growth by sulphate of ammonia on the A subplots might have led to a measure of depletion of the root reserves without causing any noticeable diminution in the number of rootlets, and a much more elaborate and careful examination of the root systems on a quantitative basis would be necessary before trustworthy conclusions on this aspect of the problem could be drawn.

## Conclusions

The conclusion may be drawn that the artificial stimulation of growth over a period of years by the use of sulphate of ammonia had reduced the inherent vigour of the grasses, so that they were unable, when the stimulus was no longer administered, to display as quick growth as grasses which had not been subjected to such stimulation. That recovery is possible, however, is shown by the results in Table II, for in 1936 (third season following discontinuance of sulphate of ammonia applications), the A subplots still not receiving sulphate of ammonia (A 1, A 8 and A 10) averaged a yield of dry matter almost the same as that of the B and C subplots. At this stage, moreover, the grasses in the A subplots were able to respond, as in the earlier years, to dressings of sulphate of ammonia, the use of which on subplots A 3, A 5 and A 12 restored these areas to their original pre-eminence from the standpoint of yield.

It may also be pointed out that the original dressing of 2 tons of ground limestone per acre applied to the A, B and C subplots in 1929 was far from being exhausted at the end of the trials in 1937. The soil from all

the subplots (including D 13, which did not receive the limestone dressing) still displayed a faintly alkaline reaction to bromthymol blue and showed effervescence when treated with hydrochloric acid.

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# NUTRITIVE VALUE OF PASTURE

## XIV. THE INFLUENCE ON YIELD AND COMPOSITION OF A SINGLE HEAVY DRESSING OF SULPHATE OF AMMONIA COMPARED WITH THAT OF PERIODIC SMALL DRESSINGS THROUGHOUT THE SEASON

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### Introduction

This paper deals with the final phase of the investigations described in the two papers immediately preceding it in the present issue of this *Journal*. These earlier papers may therefore be consulted for complete details concerning the general design and wider issues of the work as a whole (Woodman & Evans, 1938*a*, *b*).

The first three years of these grassland investigations (1929, 1930 and 1931) had been restricted to ascertaining the effect on yield and composition of the application of small dressings of sulphate of ammonia at intervals throughout the season. The question naturally arose as to whether a single heavy dressing applied early in the year would achieve the same effect in respect of yield improvement as the same amount of sulphate of ammonia applied in a number of small dressings at stated intervals. The work carried out during the seasons of 1932 and 1933 was designed to throw light on this aspect of the problem.

The six A subplots were divided into two groups of three, each group including one A subplot from each of the sections into which the subplots had been divided for convenience of cutting (Woodman & Evans, 1938*a*). During 1932, subplots A1, A5 and A10 received sulphate of ammonia at the rate of 3⅛ cwt. per acre on 16 February. On the same day subplots A3, A8 and A12 were given sulphate of ammonia at the rate of only ⅝ cwt. per acre, these subplots also receiving small dressings at this rate during the following March, April, May and July, the total application amounting therefore to 3⅛ cwt. per acre. This might nowadays be considered an unduly heavy dressing for grassland, but at the date of this investigation, when enthusiasm for nitrogen on pastures was running high, it was not regarded as exceptional. It must also be borne in mind

that an exaggeration of the conditions considered normal in ordinary practice is sometimes advisable in exploratory work in order to increase the likelihood of arriving at a clear-cut result.

The treatment of the two groups of A subplots was reversed during 1933, when subplots A3, A8 and A12 received the single heavy dressing of 3½ cwt. per acre on 15 February, whilst subplots A1, A5 and A10 were given the periodic dressings of ⅝ cwt. per acre of sulphate of ammonia on 15 February and during the following March, April, May and July.

## RESULTS OF TRIALS

The yield results, under the system of monthly cuts during the two seasons, are recorded in Table I together with figures for other years in which the two groups of A subplots received identical manurial treatment.

Table I. *Average yields of herbage from the A subplots*

| Season | Treatment | Group 1<br>A1, A5, A10<br>lb. D.M. per acre | Group 2<br>A3, A8, A12<br>lb. D.M. per acre |
|---|---|---|---|
| 1929 | Periodic small dressings of N to all A subplots | 3996 | 3969 |
| 1930 | ,, ,, | 8031 | 7961 |
| 1931 | ,, ,, | 8143 | 8404 |
| 1932 | Single heavy dressing of N to subplots in group 1<br>Periodic small dressings of N to subplots in group 2 | 6090 | 6714 |
| 1933 | Periodic small dressings of N to subplots in group 1<br>Single heavy dressing of N to subplots in group 2 | 5069 | 5228 |
| 1934 | No applications of sulphate of ammonia | 3040 | 2960 |
| 1935 | ,, | 4151 | 3893 |

### *Comments on Table I*

During 1932 the A subplots receiving sulphate of ammonia in the form of periodic small dressings throughout the season averaged a distinctly heavier yield of herbage than those which received the whole of the nitrogen in one single dressing during February. This result was not confirmed, however, in the following season, when the subplots receiving the single heavy dressing gave the heavier yield, although the difference in this case, when compared with the differences given in Table I for other years in which the A subplots in both groups received identical treatment, cannot be regarded as having real significance.

It is necessary to explain, however, why the effect noted in 1932 was reversed in 1933, and to do this, the differing weather conditions in the two years must be taken into account. At the time of applying the February dressing of sulphate of ammonia in 1932, the grass was very wet following the thaw of the previous night's frost. There was no rain in

the following days, the weather remaining cold with a number of night frosts. Under these conditions, during the week following the application, the heavily dressed subplots turned dark brown to almost black in colour and had the appearance at a distance of having been heavily scorched by fire. The lightly dressed subplots, on the other hand, were scarcely affected.

Despite the continuance of cold, dry weather, the heavily dressed subplots were beginning to show a very distinct recovery by the beginning of March, signs of the return of greenness being very apparent. Rain came on 7 and 8 March to aid recovery, and by 22 March, when the first cuts were taken, the "burnt" appearance had gone entirely. Recovery was so complete that the herbage, in which no wild white clover could be seen, was a much deeper green than on any of the other subplots. It may be noted here that clover did not begin to make its appearance again in any considerable amount on the heavily-dressed subplots until the following June.

The grass in the first cut from the heavily dressed subplots clearly consisted of new growth, the winter grass having been lost as a result of the "scorching" effect of the sulphate of ammonia. These subplots averaged 960 lb. of dry matter per acre from the first cut as against 1072 lb. in the case of the lightly dressed subplots, in which the winter growth had not been disadvantageously affected. This initial advantage was still in evidence at the time of the "flush", the heavily-dressed subplots yielding at the rate of 1785 lb. of dry matter per acre during May as compared with 1869 lb. for the lightly dressed subplots.

Thus, owing to the early "scorching" effect of the heavy application of sulphate of ammonia, the subplots receiving this dressing had been unable to gain that initial advantage of growth which should otherwise have been manifested as a result of the presence in the soil of an abundance of soluble nitrogen. During the rest of the season, the lightly dressed subplots began to respond to the periodic small dressings of sulphate of ammonia, and under their stimulus went still further ahead in respect of yield. During July and August, for example, they yielded from the two cuts at the average rate of 1280 lb. of dry matter per acre, compared with 1067 lb. in the case of the heavily-dressed subplots.

The weather conditions in the following year were quite different. There had been no frost during the night prior to the February application of sulphate of ammonia, and the grass was almost free from superficial moisture. Little or no "browning" of the herbage was noted on the heavily dressed subplots this year. The exceptionally warm and sunny

March was extremely favourable to early growth and enabled these subplots to take full advantage of the presence of the abundant supplies of soluble nitrogen in the soil. By the date of the first cut (21 March) they stood out clearly as having the thickest and deepest green growth of herbage. They averaged 1527 lb. of dry matter per acre from the first cut, thus gaining a large initial advantage over the lightly dressed subplots, which averaged only 1061 lb.

During a normal summer, the lightly dressed subplots would have been expected, under the stimulating influence of the periodic small dressings of sulphate of ammonia, to have regained the ground lost during the early part of the year. It might even have been expected that the vigorous stimulation of early growth on the heavily dressed subplots would have influenced adversely the productivity of these areas in the later part of the season because of depletion of root reserves, a circumstance that would have assisted the lightly dressed subplots to make up the lost ground. The summer proved unfavourable, however, from the standpoint of such comparisons; it was abnormally hot and droughty, a season in which dressings of artificial fertilizers such as sulphate of ammonia could not be expected to exert any outstanding stimulating effect on yield. Thus, although the extremely favourable meteorological conditions of the very early part of the season had enabled the heavy February dressing to display its full effect on the yield of early growth, the conditions in the later period of the season prevented the periodic small dressings from exerting their stimulation of productivity on the usual scale. In this manner, therefore, the weather conditions of 1933 were responsible primarily for the reversal of the effect noted in the previous year.

It was found also that an initial heavy dressing of sulphate of ammonia may have a profound influence on the crude protein content of the early cut grass. During 1932, for example, the first cut from the heavily dressed subplots contained, on the basis of dry matter, 30·6 % of crude protein. It is of interest to note that this cut was entirely grassy in character and that its richness in protein could not in the slightest degree be attributed to the presence of clover leafage. The lightly dressed subplots yielded a first cut that contained a much lower percentage of crude protein, namely, 22·8 % on the dry-matter basis. The corresponding values for the second monthly cut were 25·0 and 22·7 % respectively, but during the rest of the season the values for the herbage from the two groups of A subplots showed only very slight differences. The results for the following year were entirely confirmatory, the first cut from the heavily

dressed subplots containing, on the basis of dry matter, 25·0 % of crude protein, compared with 19·7 % in the case of the lightly-dressed subplots.

## Conclusions

It has been shown that a heavy dressing of sulphate of ammonia applied to grassland in February may cause, if the weather conditions are of a suitable character, a very pronounced stimulation of early growth of unusually high crude protein content. On the other hand, if unfavourable conditions, such as were experienced at the time of the February dressing in 1932 and are always liable to be encountered at this period of the year, should be prevalent during the week in which the heavy dressing is applied, very severe "scorching" of the herbage may result and lead to widespread "blackening" of the pasture. Recovery may be quick enough, however, to give a good growth of protein-rich herbage by the beginning of April under the conditions of southern England, but the yield may be smaller than might have been obtained if the pasture had received only a light dressing of sulphate of ammonia. This lowered productivity may persist right up to the time of the "flush" growth.

For efficient utilization of an early heavy dressing of sulphate of ammonia, the weather conditions at this period of the year must be specially favourable, as in 1933, so as to enable the herbage plants to take full advantage of the abundant supply of soluble nitrogen in the soil, since it is only over this restricted period that such a dressing is able to exert its stimulating effect on the growth of the grass.

A light dressing of sulphate of ammonia during February has the advantage of encouraging early growth without risk of "scorching" the herbage. Moreover, the additional light dressings applied at intervals during the summer have the effect of stimulating growth at times when grass normally tends to be scarce, whereas the influence of the single heavy dressing applied in February does not extend to these later stages of the season.

Since no farmer could view with equanimity the possibility of extensive "scorching" of his pastures, even though he could be assured that recovery, as in the present trial, would be prompt, it must be concluded that the method of applying sulphate of ammonia in a number of moderate dressings spread over the season is to be preferred to that of applying it all at once in a single heavy dressing during February. Whether, however, it would be desirable in practice to divide the total application of sulphate of ammonia into as many as five separate dressings is, on the score of labour, very doubtful. The determining factor must be the finding

that such added nitrogen exerts its greatest effect on yield at those times of the season when the grasses are growing most actively (Woodman & Underwood, 1932). The best method, therefore, would be to give a moderate dressing in February to encourage earliness of growth; another in late April to increase the rate of growth during the "flush" period of late May and a third and perhaps smaller application during July to stimulate the secondary "flush" usually experienced in August. It should be pointed out, of course, that these dates refer only to the conditions of the southern half of England.

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# INVESTIGATIONS INTO THE ENVIRONMENT AND NUTRITION OF THE CULTIVATED MUSHROOM (*PSALLIOTA CAMPESTRIS*)

## II. THE EFFECT OF CALCIUM AND PHOSPHATE ON GROWTH AND PRODUCTIVITY

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### Introduction

In a previous paper (Pizer, 1937) it has been shown that the physical state of composted horse manure is of great importance as regards the growth of the mushroom mycelium. A granular condition obtained by flocculating the manure with small amounts of calcium—0·5 part of calcium per 100 parts of dry compost, wt./wt.—is most suitable for rapid, vigorous growth, while a dispersed greasy condition arising through a shortage of calcium is very unfavourable and may prevent growth entirely. In both flocculated and dispersed composts, mycelial growth is made more vigorous by small additions of a soluble phosphate, equivalent to 0·031 g. phosphorus per 100 g. of dry compost. Further experiments dealing with the practical application of these results are described in the present paper. The calcium compounds used were commercial flake calcium chloride (26),[1] ground gypsum (24), superphosphate of lime (21) and hydrated lime (53), the latter being used in error for ground carbonate of lime (38). All these materials, except hydrated lime, were found to be satisfactory in laboratory trials for promoting mycelial growth, but the best results were given by the first three. The materials differ greatly in composition and properties, in ease of application and reactivity, and probably in their influence on the metabolism and productivity of the mushroom, and it was the main object of the experiments to discover the most suitable one for the grower to use.

Composts were made from fresh horse manure and wheat-straw bedding, obtained from the College farm and from army stables. For the purpose of the experiments, the manure from both these sources was

[1] The figures in brackets indicate the percentage of calcium in the materials.

excellent, since in the past it had shown a marked tendency to become wet and greasy during composting even when great care was taken in watering and turning it, and on the composted manure mycelial growth and cropping had been patchy and poor. Composting was carried out under cover in heaps 4 ft. high by 6 ft. wide, containing 4–5 tons of manure and was usually completed in 3 weeks, after 4–5 turns at intervals of 4 and 3 days. The manure usually heated well in the heaps but reached moderate temperatures only (90–110° F.) in the beds.

Flat beds were made, 3 ft. wide by 52 ft. long, arranged in tiers, the lowest being on the chalk floor of the house and the others on wood or corrugated-iron shelves. The compost was pressed down firmly to a depth of 6–7 in. and four days later spawned at intervals of 10 in. with fresh, moist, pure-culture spawn of a white variety. Since the presence of the casing soil prevents proper examination of mycelial growth, the beds were first covered with straw and not cased until three weeks after spawning. A clay loam subsoil, with *p*H 7·5, dug in good granular condition was used for casing. Cropping was delayed by late casing but the first picking was usually obtained 3–4 weeks after casing.

The experiments were carried out in the summer months when day temperatures in the house were frequently between 70 and 80° F. and the humidity was low, and these conditions resulted in many small light mushrooms and short cropping periods. Some of the beds were attacked and severely damaged by the mite, *Linopodes motatorius* L., and by the larvae of phorid and sciarid flies.

## FIRST SERIES OF EXPERIMENTS

In these experiments a number of calcium compounds were mixed as thoroughly as possible with the compost as the beds were being made. It was found that the quantities which acted satisfactorily in laboratory trials were too small to be applied evenly and that to obtain proper distribution, which is one of the main factors in flocculation, relatively large quantities were required. Since mineral matter in large quantities may be detrimental (Styer, 1930), it was decided to find out whether damage resulted from very heavy applications; superphosphate was applied more lightly than the other minerals on account of its greater solubility. Calcium chloride was not used since it must be applied in solution and, as a rule, wetting of the beds is undesirable. The experimental plots were in groups of four, well away from the ends of the beds and each had an area of 30 sq. ft. (3 × 10 ft.). The results and other data are summarized in Table I and brief notes on each experiment are given below.

*Exp. I.* The compost was prepared from strawy manure in which the droppings were doughy in texture and failed to break apart readily when struck with a fork. Under pressure in the compost heap a large proportion of the droppings flattened out or bound the straw into greasy lumps. Plots on a single shelf were treated with ground gypsum, superphosphate, nothing and hydrated lime respectively. On the gypsum and superphosphate plots the compost and casing soil were thoroughly and strongly run with mycelium but on the untreated and hydrated lime plots, large areas were not attacked; mycelial growth was patchy and largely in the top two inches of the manure and was very poor in the casing soil. On no plot was growth rapid, and all plots were slow coming into bearing. The gypsum and superphosphate plots began cropping well but were severely damaged by *Linopodes motatorius* L., and the yields were low.

*Exp. II.* A second bed was filled with compost from the heap used in Exp. I and the first three plots were treated with gypsum, nothing and gypsum respectively, while on the fourth plot gypsum was scattered on the surface only. Mycelial growth was poor on the untreated plot and good where gypsum was mixed with the manure; on the fourth plot, growth was strong in the surface but poor and irregular below two inches. Yields were better on the treated plots but were reduced by sciarid larvae which destroyed mycelial tissue and tunnelled the mushrooms, reducing their weight.

*Exp. III.* The compost was made from well-balanced manure and was lumpy but less greasy than that used in previous experiments. The experiment resembled Exp. I, but in this case the plots were not damaged by mites, and growth of the spawn and yields on corresponding plots were better. On the gypsum and superphosphate plots the run was very strong and dense both in the compost and in the casing soil. On the untreated plot the run was moderate and thin in places but not patchy and was much better than on the hydrated lime plot.

*Exp. IV.* The manure was rich in droppings and became lumpy and very greasy during composting. Two plots were treated with gypsum and two were untreated. The spawn ran slowly but evenly into the treated beds without attacking the manure strongly and an even thick crop of mushrooms was produced; the beds were severely damaged by mites and phorid larvae and the yield greatly reduced. On the untreated beds the spawn grew hardly at all and in places failed completely.

*Exp. V.* The fresh manure contained a high proportion of straw and the droppings were slightly greasy, whole, compact and difficult to break

apart. The compost was greasy and lumpy and contained plenty of unbroken droppings. The treatments were the same as in Exps. I and III. Growth was slow in all cases; where gypsum and superphosphate were added growth was best, but parts of the compost which they did not enter were untouched. The yields were poor except on the gypsum plot and appeared to be depressed on the superphosphate plot.

*Exp. VI.* The fresh manure and compost were similar to those in Exp. V. Two plots were treated with gypsum and two were untreated. The spawn ran well in the plots containing gypsum but the crop was greatly reduced as a result of phorid and sciarid attack. On the untreated manure there was hardly any growth and very few mushrooms.

### SECOND SERIES OF EXPERIMENTS

In these experiments, commercial flake calcium chloride or ground gypsum were added to the fresh manure as the first composting heap was made; 2 lb. of 70 % calcium chloride or 28 lb. of ground gypsum were added per ton of manure, the quantities being chosen in the following way:

In laboratory trials (Pizer, 1937) an $M/40$ solution of calcium chloride and 1·25 to 1·9 parts of gypsum per 100 parts of dry compost (wt./wt.) flocculated *composted* manure very effectively, which quantities are equivalent to 2 lb. of 70 % calcium chloride and 6·5–9·5 lb. of gypsum per ton of *fresh* manure respectively, assuming that composted manure contains 55 % moisture and that half the moist weight of manure is lost during composting (Waksman and Nissen, 1932). The calcium chloride is readily distributed by dissolving it in any suitable volume of water, but to obtain even distribution of the gypsum it must be increased to 28 lb. per ton of manure.

The arrangement of experimental plots was the same as in previous experiments.

*Exp. VII.* The fresh manure resembled that in Exp. IV in condition. Ground gypsum was applied to it at the rate of 28 lb. per ton of manure and at the first turn the droppings broke apart readily as the manure was shaken out. Composting was comparatively rapid, four turns only being necessary; the compost contained very few whole droppings, most of them being in small fragments well scattered throughout the straw, and it was granular in texture. Two plots were given a light dressing of superphosphate and two were untreated. The mycelium grew very well in all the plots but where superphosphate was used the growth was better,

Table I. *First series of experiments. Yields per sq. ft. of bed surface*

| Experiment no. | Treatment | Spawning to cropping days | Duration of cropping days | Yield first 21 days oz. | Yield second 21 days oz. | Yield in final period oz. | Total yield oz. | Application per sq. yd. lb. | Application per ton of compost lb. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| I | Ground gypsum | 53 | 44 | 7·4 | 3·3 | 0·4 | 11·1 | 3·1 | 48 | Severe mite damage |
| . | Superphosphate | 53 | 44 | 10·2 | 5·3 | 0·6 | 16·1 | 1·2 | 18 | ,, |
| | Untreated | 55 | 46 | 4·9 | 6·5 | 1·1 | 12·5 | — | — | |
| | Hydrated lime | 55 | 48 | 3·8 | 4·1 | 0·7 | 8·6 | 2·7 | 42 | |
| II | Ground gypsum | 53 | 53 | 4·5 | 9·7 | 3·3 | 17·5 | 7·2 | 110 | Severe sciarid damage |
| | Untreated | 55 | 42 | 5·2 | 7·9 | 0·7 | 13·8 | — | — | ,, |
| | Ground gypsum | 55 | 53 | 7·9 | 13·6 | 2·5 | 24·0 | 5·9 | 91 | ,, |
| | Gypsum on surface | 53 | 55 | 6·4 | 7·2 | 4·2 | 17·8 | — | — | |
| III | Hydrated lime | 55 | 59 | 2·8 | 6·8 | 2·0 | 11·6 | 2·3 | 35 | |
| | Superphosphate | 48 | 66 | 5·5 | 12·3 | 4·6 | 22·4 | 1·6 | 25 | |
| | Untreated | 57 | 57 | 8·0 | 8·2 | 2·3 | 18·5 | — | — | |
| | Ground gypsum | ·57 | 68 | 11·9 | 11·3 | 4·1 | 27·3 | 3·2 | 50 | |
| IV | Untreated | 50 | 59 | 0·5 | 0·2 | 1·8 | 2·5 | — | — | |
| | Ground gypsum | 50 | 59 | 5·6 | 2·2 | 8·1 | 15·0 | 3·0 | 45 | Severe mite and phorid damage |
| V | Ground gypsum | 57 | 62 | 7·1 | 8·7 | 3·8 | 19·6 | 5·8 | 89 | |
| | Untreated | 64 | 55 | 4·9 | 6·3 | 0·9 | 12·1 | — | — | |
| | Hydrated lime | 61 | 58 | 4·2 | 8·0 | 1·6 | 13·8 | 3·0 | 46 | |
| | Superphosphate | 60 | 59 | 6·2 | 4·9 | 1·3 | 12·4 | 3·5 | 53 | *Yield depressed?* |
| VI | Untreated | 59 | 59 | 2·3 | 2·7 | 1·7 | 6·7 | — | — | *Phorid and sciarid damage* |
| | Ground gypsum | 59 | 59 | 10·7 | 2·7 | 2·3 | 15·7 | 4·4 | 67 | Severe phorid and sciarid damage |

the compost was strongly and thoroughly attacked, the casing soil was filled with strands and a yield of more than 2 lb. per sq. ft. was obtained.

*Exp. VIII.* Half the fresh manure used in Exp. V was watered with a solution of calcium chloride, using 2 lb. calcium chloride per ton of manure. It composted more rapidly than the untreated manure and the droppings gradually broke apart; the compost was uniform in appearance but slightly greasy. The plots were treated with ground gypsum, nothing, hydrated lime and superphosphate respectively. Except on the plot receiving hydrated lime where growth was poor, the spawn ran uniformly to the bottom of the beds (cf. Exp. V). The mycelium was stronger and denser, and yields were better on the gypsum and superphosphate plots than on the untreated plot.

*Exp. IX.* Half the fresh manure used in Exp. VI was treated with ground gypsum at the rate of 28 lb. per ton of manure. Composting was more rapid in the treated heap; at the second turn the droppings were thoroughly broken up and mixed with the straw, and the composted manure was uniform and granular (cf. Exp. VI). Two plots were given a light dressing of superphosphate and two were untreated. The mycelium ran uniformly to the bottom of the plots but it was stronger and denser where superphosphate was added. The crop was probably reduced by exposure to draughts and dryness of the casing soil which became dry soon after watering. Yields were slightly better on the superphosphate plots.

*Exp. X.* A number of beds were made from manure composted with gypsum and superphosphate. The dressings per ton of fresh manure were 28 lb. of gypsum at the beginning of composting and 28 lb. of a mixture of equal parts by weight of gypsum and superphosphate at the last turn. The mycelium grew strongly but yields were reduced by mite injury. An average yield of 18 oz. per sq. ft. was obtained.

### THIRD SERIES OF EXPERIMENTS

*Exp. XI.* The previous experiments show that small quantities of superphosphate stimulate mycelial growth and have a small effect on yield. In order to measure the effect of superphosphate more precisely, a third series of experiments was carried out with plots arranged so that the yields could be examined statistically. In addition to superphosphate, ammonium sulphate was added to some of the plots, since in laboratory trials the application of ammonium sulphate to a number of composts resulted in better mycelial growth.

Table II. *Second series of experiments. Yields per sq. ft. of bed surface*

| Experiment no. | Treatment | Spawning to cropping days | Duration of cropping days | Yield first 21 days oz. | Yield second 21 days oz. | Yield in final period oz. | Total yield oz. | Application per sq. yd. lb. | Application per ton of compost lb. | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|
| VII | Untreated | 49 | 67 | 8·9 | 8·4 | 8·5 | 25·8 | — | — | |
| | Superphosphate | 49 | 67 | 15·2 | 10·6 | 9·2 | 35·0 | 0·7 | 10 | |
| VIII | Ground gypsum | 58 | 57 | 7·0 | 13·7 | 2·4 | 23·1 | 8·1 | 124 | |
| | Untreated | 57 | 58 | 7·9 | 9·3 | 3·6 | 20·8 | — | — | |
| | Hydrated lime | 62 | 53 | 4·5 | 6·6 | 0·5 | 11·6 | 5·8 | 89 | |
| | Superphosphate | 62 | 64 | 11·1 | 10·8 | 2·7 | 24·6 | 3·5 | 53 | |
| IX | Superphosphate | 57 | 48 | 11·5 | 6·4 | 0·4 | 18·3 | 0·8 | 13 | Yields reduced on all plots by dryness |
| | Untreated | 57 | 60 | 11·1 | 4·8 | 1·7 | 17·6 | — | — | |
| | Superphosphate | 57 | 56 | 15·6 | 4·6 | 0·5 | 20·7 | 0·9 | 14 | |
| | Untreated | 57 | 56 | 13·2 | 2·3 | 0·6 | 16·1 | — | — | |

The design of the experiment was based on the recommendations of Lambert (1934). The plots were on shelf beds in a house 60 × 40 × 10 ft. high to the ridge. The shelves were in tiers separated by a pathway 3 ft. wide. The tiers contained three or four shelves 2 ft. apart, 3 ft. wide and 52 ft. long. Beds in neighbouring tiers were used for the experiment, but the top shelves near the roof were excluded on account of the wide daily fluctuations in temperature and humidity which occur in these parts of a house. Each bed was divided into six plots of equal size, 6 × 3 ft. Extreme plots were 3 ft. from the ends of the shelves, and strips 2 ft. wide separated the plots from each other. The plots were grouped in blocks and the treatments randomized within the blocks. The treatments were per 18 sq. ft. of bed: (1) nothing, (2) 1 lb. of superphosphate, (3) 1 lb. of superphosphate + ½ lb. of ammonium sulphate, (4) 1 lb. of superphosphate + 1 lb. of ammonium sulphate. Treatments (1) and (2) were randomized within four blocks on two shelves situated one above the other. Treatments (2), (3) and (4) were randomized within four blocks on two shelves at the same level in neighbouring tiers. Treatment (2) was applied to six plots all on one shelf. The dressing of superphosphate was less than the smallest amount used in previous trials (cf. Exp. VII) and is equivalent to about 7 lb. per ton of composted manure or 3½ lb. per ton of fresh manure. The smaller dressing of ammonium sulphate is equivalent to about 4 lb. per ton of compost (or 2 lb. per ton of fresh manure) and is of the same order as that used by previous investigators (Demolon *et al.* 1935).

The compost was obtained from one heap and was made from College horse manure to which ground gypsum was added at the rate of 28 lb. per ton of manure at the beginning of composting. The manure composted well and the final product was uniform in appearance and slightly strawy. Previous experience had shown that College manure gave average yields of mushrooms and responded to applications of superphosphate.

The beds were spawned at intervals of 10 in. without regard to the positions of the plots. The spawn ran well on all beds and the appearance of the first "flush" and of the mycelium indicated that good yields would be obtained. Unfortunately, the beds were early attacked by the mite, *Linopodes motatorius*, and severely damaged, and as a result the highest yield given by any plot was 10 oz. per sq. ft. The mite destroyed the mycelium almost entirely and severed the mycelial strands at the base of the mushrooms.

The variation in yield within treatments and between blocks was high. This was probably due to a number of causes—differences in the compost,

in local conditions in the house, in management of the beds and in insect attack—but was attributed largely to damage by mites.

The yields were:

| Treatment | 1 | 2 | Block averages | 2 | 3 | 4 | Block averages | 2 (6 plots) |
|---|---|---|---|---|---|---|---|---|
| Mean yield oz./sq. ft. | 7·3 | 8·0 | 7·6 | 5·2 | 5·7 | 4·4 | 5·1 | 6·6 |
| Coefficient of variability | 35·2 | 30·9 | 30·7 | 46·0 | 17·2 | 33·9 | 31·5 | 35·7 |

It is obvious that definite conclusions on the effect of treatment on yield cannot be drawn from the figures but there is an indication that the higher quantities of ammonium sulphate depressed the yield. The amounts of both fertilizers were much higher than those commonly given to other crops—1 lb. per 18 sq. ft. ≡ 1·1 tons per acre—and better results might be obtained with smaller quantities. It is sometimes stated that fermentation and heating are renewed when ammonium sulphate is added to composted manure, but this did not occur in the present instance.

## Discussion

The compounds used in the above experiments bring about changes in the chemical composition, in the physico-chemical properties and in the structure of composted manure and, as a result, the latter is altered in character as a medium and environment for living organisms. Some of the organisms occurring in composted horse manure compete with the mushroom for food and others destroy mycelial tissue, so that it is important to know how they are affected by changes in the environment, but the discussion is limited by the scope of the experiments to the mushroom only.

A treatment usually has two important effects on the mushroom—it affects the development of the mycelium and of the sporophores. There is no known method of measuring accurately changes in mycelial growth, and the effect of a treatment must be judged largely from the appearance of the mycelium. As a rough guide the extent of growth may be measured, but in some cases the principal change is in the density of mycelial growth and in the strength and appearance of the individual hyphae. Some growers like the hyphae to be so extremely fine and numerous that the mycelium has the appearance of a bluish smoke enveloping the particles of compost; others prefer the hyphae to be thicker, covering the compost with a dense white growth. It is not known whether the above forms are similar in cropping capacity, or whether the type of mycelial growth bears any direct relation to cropping, except that where the mycelium is weak and stunted, cropping is bound to be poor.

In the above experiments, yields were considerably affected by insect damage to the mycelium and to the sporophores; many mushrooms failed to develop owing to separation from the mycelial strands which fed them, and many of the large ones were light in weight owing to tunnelling of the caps and stems by sciarid and phorid larvae. Environmental conditions on the whole were more favourable for mycelial growth than cropping, temperatures as a rule being high and humidity low. In these circumstances, the effect of a particular treatment on yield is, in some cases, only to be inferred from the appearance of the mycelium and of the first flush.

As regards the effect of calcium on mycelial growth, the evidence is quite definite that on certain kinds of manure proper mycelial growth does not occur unless a calcium compound is added—see the first series of experiments.

Not all calcium compounds are suitable. Hydrated lime is harmful (Exps. I, III, VIII) and should on no account be used as small amounts may make the compost too alkaline. Calcium carbonate was not tried, but in laboratory trials it was less effective than the chloride, sulphate and acid phosphate, probably owing to its low solubility under alkaline conditions, but it is possible that alkali carbonates, formed by base exchange, increase the *p*H and have a detrimental effect on the structure of the manure. Calcium chloride is one of the best flocculants of manure but it is not readily stored and handled and, being very soluble, it may increase the osmotic pressure unduly in composts that already contain a high amount of soluble material (Styer, 1930); also, where it was used (Exp. VIII) freshly cut mushroom tissue changed in colour from white to pink in a few seconds and rapidly became brown; this effect was not observed in Exp. V where similar manure was used or in other experiments and it may possibly have been caused by the chloride ion.

Superphosphate flocculates manure very readily since it is moderately soluble in water. It is acid in reaction and lowers the *p*H, especially in manure that is very alkaline—it is used in the U.S.A. to reduce the *p*H of alkaline composts—but as composts are well-buffered in the acid range the effect is not harmful with small amounts of superphosphate. The influence of superphosphate on mycelial growth is due largely to the action of calcium on the compost and to the phosphate ion which appears to increase the number and thickness of the hyphae (see Exps. VII and IX in particular). Styer (1928) showed that mycelial gr wth is limited by lack of phosphate more than by lack of other minerals and that a relatively high concentration of phosphate is required to obtain good

growth. On the average, the phosphorus content of horse manure is low and therefore superphosphate may be of considerable benefit in some cases. In most of the experiments with superphosphate, earlier cropping and a greater number of buttons were obtained; small amounts of 5–9 lb. per ton of fresh manure increased the crop but large amounts appeared to depress the yield. Growers have reported that 14 lb. of superphosphate per ton of fresh manure (Pizer, 1937) have stimulated mycelial growth and the production of buttons and caused early cropping, but while in some cases the yield has been better, in other cases it has not been as good because the beds finished cropping earlier. In view of these reports, superphosphate should not be used as a flocculating agent but only to supply phosphate. On manure that gives unsatisfactory results it is well worth a trial and is most easily applied mixed with gypsum; 28 lb. of a mixture containing not more than 7 lb. of superphosphate may replace the second application of gypsum (see later).

Ground gypsum is an efficient flocculating agent and although slower in its action than calcium chloride it has other properties which make it the best of the materials tried. It is effective under both alkaline and acid conditions and its maximum solubility in water is so low (about $0{\cdot}015\,M$) that the osmotic pressure is not seriously affected by large applications—according to Styer (1930), the concentration of highly dissociated salts should not exceed $0{\cdot}1\,M$ when other soluble material is present. Gypsum supplies sulphate which may have nutritive value, it appears to have no undesirable effects on the metabolism of the mushroom, it is cheap and readily obtainable. The amounts of gypsum used in the above experiments ranged from 22·5 to 56 lb. per ton of fresh manure (equivalent to 45 to 112 lb. per ton of composted manure) and in all cases healthy vigorous growth and normal cropping were obtained. Even distribution can be obtained with 28 lb. of gypsum per ton of manure and in practice this appears to be a suitable amount to apply to the fresh manure. Following the results obtained in the above experiments, gypsum was recommended to growers to ensure proper mycelial growth (Pizer, 1936) and it is now widely used by them; most growers find that gypsum produces more reliable composts, but a few, who have perhaps been fortunate with their supplies of manure, consider that gypsum is not necessary.

The value of gypsum lies largely in its flocculating action and in other physico-chemical changes produced by calcium in the manure. Gypsum may be applied to the fresh or to the composted manure or at any time during composting, but the earlier it is applied the better is the effect.

When applied to the fresh manure, distribution is more thorough since remixing with the manure occurs each time the heap is turned and the rate of diffusion of calcium into the manure is greater owing to the wet state of the manure. When applied to the composted manure as the beds are made, flocculation occurs locally where the gypsum is in contact with the compost and, owing to the small amount of free water, diffusion of calcium into other parts is slow or negligible, as witness the patchy growth on treated plots in Series I and the surface growth in Exp. II, where gypsum was scattered on the surface of the bed only.

The physico-chemical changes produced by calcium are of great value during fermentation. The capacity of the colloids to imbibe and hold water is increased and the flocculated manure has a more rigid, granular structure. As a result, drainage and aeration in the manure are improved and it is possible to add considerably more water without the manure getting into a sodden and compact state. Aerobic fermentations are favoured and, judged by the smell of the composted manure, products of anaerobic fementation accumulate to a less extent. With more water in the manure and better aeration, chemical changes are more rapid and the manure composts more quickly. As a result of flocculation, the droppings break up as the manure is turned and mix readily with the straw, and the final compost is a uniform product in good physical condition and without an unpleasant smell.

Some growers delay the application of gypsum until the last turn because it makes the manure drier, thus allowing greater latitude with watering during the final stages of composting—the drying action of gypsum is due to physico-chemical changes in the manure in which water is withdrawn from the free state and bound by the colloids. The advantage is more apparent than real because it is much easier to control the wetness during composting when the manure contains gypsum. In some cases, mycelium grows very slowly in manure that is flocculated after composting; the reason for this is not known but it may be due to products of anaerobic fermentation which, though not entirely eliminated, are not formed to the same extent when gypsum is added at the beginning of composting.

The most satisfactory results with gypsum are obtained when 28 lb. are applied per ton of fresh manure when the first composting heap is made. If towards the end of composting, the manure is noticeably greasy or doughy in texture or contains whole droppings that do not break readily, which sometimes happens when the proportion of droppings in the manure is high, a further 28 lb. of gypsum per ton of manure should

be applied at the last turn; some growers make two applications of gypsum in the above manner as a matter of routine to be on the safe side but usually one is ample.

For many years in the mushroom growing region around Paris, some growers have mixed 45 lb. of burnt gypsum (plaster of Paris)—equivalent to 56 lb. of gypsum—with each ton of *composted* manure when the beds are made (Demolon *et al.* 1935) to control the moisture content of the compost and to facilitate moulding of the beds (Burgevin, 1937) (ridge type). French literature appears to contain no reference to the first use of burnt gypsum or the reason for using it (Burgevin, 1937). A few growers in this country and in the U.S.A. (Lambert, 1932) have used gypsum or burnt gypsum for some time, possibly on the recommendation of French spawn makers.

It is possible that gypsum is of nutritional value to the mushroom. From analytical work on the composition of the ash of mushrooms and of composted horse manure, Hébert & Heim (1909, 1911) concluded that fertilizers containing calcium and sulphate should be added to the manure since the mushroom appeared to require large amounts of the radicals and the quantities in manure were not very high. Later, however, Styer (1928) showed that the mushroom mycelium will grow strongly when the concentrations of calcium and sulphate are extremely low, and in Exp. II above, where the mycelium had ample calcium and sulphate to draw upon, it grew only where the compost was flocculated.

There seems to be little doubt that the value of gypsum is due to changes produced by calcium ions in the physico-chemical condition of the manure of which flocculation is the most obvious. Flocculation brings about improved aeration and this may be partly responsible for better mycelial growth, but in cases of failure that are remedied by flocculation, the mycelium grows no better on the surface of the manure than in it and in some cases does not grow at all. As fresh surfaces are formed during flocculation, it is possible that these surfaces are more suitable for the progress of reactions which may be initiated by surfaces on the hyphae or by enzymes secreted by them, since the structure, composition and energy of surfaces and rates of diffusion across them play very important roles in enzyme reactions.

In a previous paper (Pizer, 1937) the mineral composition of horse foods and bedding has been mentioned as the probable cause of greasiness in composts, since in many cases the ions causing dispersion (Na, K and Mg) are much in excess of calcium. In a few instances of success and failure it has been possible to obtain information about the rations. Where good crops were grown the rations contained clover hay, beans, oats and a

little maize and the ratio[1] of the sum of the dispersing ions (Na + K + Mg) to calcium in the rations ranged from 1:4 to 1:7; where failures were obtained the rations contained meadow hay, oats and a high proportion of maize and the above ratio ranged from 2:4 to 2:8. Though the evidence is meagre it is suggested that when this ratio in a ration exceeds 1·7 then greasy mannre is likely to be obtained.

It is not known whether the growth of other organisms in composted manure is affected by flocculation of the manure with gypsum, but it is possible, as some growers maintain, that competition from white plaster mould (*Monilia fimicola*) is reduced. White plaster mould appears to predominate when the physical condition of the manure is unsuitable for the mushroom. Lambert (1932) states that a greasy compost favours the development of "plaster mould" and recently Beach (1937) has shown that white plaster mould predominates as the reaction becomes increasingly alkaline, i.e. as the compost becomes more dispersed.


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[1] To obtain this ratio the percentage of each cation was divided by its equivalent weight and the sum of the quotients for Na, K and Mg divided by the quotient for Ca.

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# THE EFFECT OF TIME AND RATE OF APPLICATION OF NITROGEN FERTILIZERS ON THE YIELD OF WHEAT

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## Introduction

Although it is well known that application of nitrogenous fertilizers to wheat often results in considerable increases in yields of grain and straw, and while experiments at Rothamsted and Cambridge have been very valuable not only in demonstrating the magnitude of the yield increases but also in showing how nitrogenous fertilizers bring about these increases, there is singularly little experimental evidence available regarding the increases in yield which may be expected in other parts of the country. Further, there is very little experimental evidence as to the optimum time of application of nitrogen, except at Rothamsted and Cambridge, both of which are in relatively dry districts.

Whilst considerable difference of opinion (*Rothamsted Ann. Report*, 1927–8; Doughty *et al.* 1929) existed several years ago regarding the *modus operandi* of nitrogen applied in early spring, it now seems clear that nitrogen applied at this time can increase yields by increasing the size of the ears and by increasing the number of ears (Russell, 1932; Garner & Sanders, 1936). The results of a pot culture experiment described by Watson (1936) show that early applications of nitrogen increase yields of grain largely by increasing the number of ears and that late applications of nitrogen increase the number of grains per ear and the 1000 grain weight but, in contrast with the "critical period" hypothesis of Doughty *et al.* (1929), Watson found that late applications of nitrogen brought about small increases in the number of ears.

It is generally assumed that nitrogen applied in the autumn is not, on an average, as effective as nitrogen applied in spring, owing to loss of nitrates by winter leaching. Fisher (1924) has shown, from a study of the results of the Broadbalk plots at Rothamsted from 1854 to 1918, that the yield of wheat is decreased in proportion to the excess of winter rainfall over the average. "Alumnus" (1932), however, has shown that spring and

summer rainfall were much more detrimental than winter rainfall to the autumn-dressed plots in Broadbalk, and that in dry summers autumn dressings were superior to spring dressings. He suggests that the plants on the autumn-dressed plots have a better chance of establishing a good root system, so that they are better able to withstand dry summers, but he points out that this is probably not a complete explanation.

The results of Doughty *et al.* (1929) and of Garner & Sanders (1936) indicate that a small amount of nitrogen applied in the autumn may be very beneficial. The possibility that autumn nitrogen may be particularly useful when "continuous" cereal growing is practised will be obvious.

In addition to the effect of nitrogen on the yield of grain, its effect on the lodging propensities of the crop is also important. Experiments at Rothamsted in 1925–8 indicated that nitrogen applied after tillering had "ceased" gave as good increases in yields of grain but less straw than early (March) applications, and it may be argued that less straw means less liability to lodging.

The aim of this paper is to present results of experiments conducted at Jealott's Hill in 1929–35 and on outside farms in 1933–5. These experiments were undertaken primarily to determine the effect, on yield and liability to lodging, of the application of nitrogen at different times (autumn, early and late spring) and at different rates, but some developmental data were also obtained. It so happened that lodging occurred in only one out of twenty-eight experiments, and the evidence on the effect of nitrogen on lodging is, therefore, largely indirect.

## EXPERIMENTAL

All the results quoted in this paper are for experiments in which each treatment was replicated at least four times. The size of the plots varied in different experiments, but single plots were never more than 1/40th or less than 1/80th acre in area. In all cases the nitrogenous fertilizers were applied broadcast by hand.

## EARLY AND LATE SPRING TOP DRESSING

### *Experiments at Jealott's Hill*

The results obtained at Jealott's Hill during 1929–35 are given in Tables I and II. The 1929 figures show that applying nitrogen on 9 May gave as high a yield of grain but less straw than did the March application. Further delay of nitrogen application until 4 June led to a much lower yield of straw, and the yield of grain was also lower; it should be noted

that June 1929 was rather dry. In 1930, the 19 May application was best from a practical point of view; the low yield of straw with this treatment was largely due to the straw being short, and this was a very definite advantage since those plots were less lodged by thunderstorms than were the plots which received their nitrogen earlier. This is in contrast to the results of Watson (1936), who found that the height of the main shoot was not significantly affected by time of application of nitrogen, but it should be borne in mind that in Watson's experiment the moisture content of the soil was maintained at a good level whereas, in the field, soil moisture content decreases as spring advances. The failure of the 12 March application to increase the yield of grain was probably indirectly due to lodging, since the crop shed some of its grain and was attacked by birds.

The 1931 and 1932 results show no appreciable differences in yields of grain or straw due to differences in time of application of nitrogen. The height of straw in 1932 was also about the same for all nitrogen treatments and it would appear that the higher yields of straw with the double

Table I. *Top-dressing experiments: Jealott's Hill*, 1929–32

*2 N = 2 cwt. nitrochalk per acre*

| | Treatment | Date of application | Grain cwt. per acre | Straw cwt. per acre | Height of straw in. |
|---|---|---|---|---|---|
| 1929 | No N | — | 21·1 | 31·2 | — |
| Variety: | 2 N "early" | 26 March | 25·5 | 41·3 | — |
| White Victor | 2 N "medium" | 9 May | 25·8 | 38·5 | — |
| Previous cropping: | 2 N "late" | 4 June | 24·1 | 33·3 | — |
| fallow | | Standard error | 0·43 | 0·74 | — |
| 1930 | No N | — | 33·0 | 54·0 | — |
| Variety: | 2 N "early" | 12 March | 33·4 | 72·5 | — |
| White Victor | 2 N "medium" | 16 April | 35·9 | 67·7 | — |
| Previous cropping: | 2 N "late" | 19 May | 37·1 | 60·9 | — |
| oats | | Standard error | 0·92 | 1·35 | — |
| 1931 | No N | — | 22·6 | 43·5 | — |
| Variety: | 2 N "early" | 25 February | 25·8 | 56·8 | — |
| White Victor | 2 N "medium" | 13 April | 25·5 | 53·6 | — |
| Previous cropping: | 2 N "late" | 18 May | 26·9 | 53·1 | — |
| beans | 1 N "early" + 1 N "late" | 25 February 18 May | 27·5 | 56·0 | — |
| | | Standard error | 1·31 | 2·60 | — |
| 1932 | No N | — | 22·2 | 39·8 | 45·0 |
| Variety: | 1 N "early" | 18 March | 25·3 | 48·1 | 49·5 |
| White Victor | 2 N "early" | 18 March | 28·5 | 52·4 | 48·8 |
| Previous cropping: | 1 N "late" | 18 May | 23·7 | 45·0 | 49·3 |
| barley | 2 N "late" | 18 May | 28·3 | 53·2 | 49·0 |
| | ½ N "early" + ½ N "late" | 18 March 18 May | 25·8 | 48·5 | 48·5 |
| | 1 N "early" + 1 N "late" | 18 March 18 May | 29·5 | 55·0 | 50·3 |
| | | Standard error | 1·20 | 2·34 | — |

Table II. *Top-dressing experiments: Jealott's Hill*, 1933–5

2 cwt. nitrochalk per acre applied to all except no-nitrogen plots

| | Date of application | Grain cwt. per acre | Straw cwt. per acre | Height of straw in. | No. of ears per yard row |
|---|---|---|---|---|---|
| 1933 | No N | 20·2 | 30·0 | 40·2 | — |
| Variety: White Victor | 21 March | 24·9 | 40·5 | 44·8 | — |
| Previous crop: Cocksfoot hay | 4 April | 26·5 | 41·4 | 45·2 | — |
| | 18 April | 25·6 | 40·8 | 43·2 | — |
| | 2 May | 24·6 | 38·2 | 44·2 | — |
| | 16 May | 24·6 | 35·8 | 41·6 | — |
| | 29 May | 23·1 | 32·4 | 41·2 | — |
| | 12 June | 23·3 | 33·4 | 41·0 | — |
| | Standard error | 0·93 | 1·38 | — | — |
| 1934 | No N | 27·3 | 36·6 | 35·8 | — |
| Variety: White Victor | 7 March | 28·7 | 41·2 | 38·0 | — |
| Previous crop: Seeds | 21 March | 30·1 | 41·4 | 38·2 | — |
| | 3 April | 29·5 | 40·9 | 37·2 | — |
| | 18 April | 30·2 | 41·9 | 36·6 | — |
| | 3 May | 29·4 | 38·0 | 37·2 | — |
| | 16 May | 29·6 | 38·0 | 35·8 | — |
| | 30 May | 30·5 | 38·3 | 37·2 | — |
| | Standard error | 0·94 | 1·33 | — | — |
| 1935 | No N | 19·4 | 27·4 | 37·0 | 41·0 |
| Variety: Squarehead's Master | 20 March | 25·7 | 46·3 | 47·4 | 47·0 |
| Previous crop: Seeds gyro-tilled | 1 April | 23·7 | 39·2 | 44·6 | 46·0 |
| | 12 April | 27·4 | 46·8 | 47·0 | 46·6 |
| | 25 April | 23·8 | 41·0 | 45·4 | 47·4 |
| | 8 May | 23·8 | 38·7 | 43·0 | 44·6 |
| | 18 May | 24·0 | 38·1 | 43·2 | 46·4 |
| | 30 May | 24·1 | 36·1 | 40·6 | 44·6 |
| | Standard error | 1·10 | 1·72 | 0·73 | 2·33 |

nitrogen dressings were due to an increase in the thickness of the straw or to an increase in the number of tillers, which must either have been "blind" or must have carried small ears.

The 1933 results indicate that the late applications of nitrogen were rather less effective than the early applications in increasing yields of grain, whilst the yields of straw were definitely lower with the late applications. In 1934, nitrogen had very little effect on the yield of grain, but the early applications increased the yields of straw without increasing its height to any marked extent. In 1935 all the nitrogen treatments gave about the same yield of grain (except 12 April dressing, the yield of which is unaccountably high), but the late dressings gave less and shorter straw than the early dressings.

The figures for numbers of ears at harvest, which are means of counts for five random yard rows per plot, show that the nitrogen treatments significantly increased the number of ears per yard row. The increase in number of ears over the control amounts, however, to only about 12%, whereas the average increase in yield of grain was about 25%. It is

therefore clear that nitrogen must also have increased ear size. Lodging occurred only in 1930, when there was definite evidence that the late dressings of nitrogen caused less lodging than did the early dressings. It can, however, be argued that late applications of nitrogen cause less liability to lodging, since they did not increase the height of straw as much as early drainage.

*Experiments at outside centres*

In the experiments at outside centres, sulphate of ammonia was used for the early dressings in order to reduce to a minimum the risk of loss of nitrogen by leaching. Nitrochalk was used for the late dressing as the best means of ensuring that part of the nitrogen would be effective whatever the weather after application; if dry, the nitrate would probably prove more effective and, if wet, the ammonia would be preferable.

Details and results of the experiments to compare early and late spring applications of nitrogen at outside centres are given in Tables III and IV respectively.

The grain figures show that:

(i) a significant increase due to nitrogen occurred at eight centres out of nine in 1933, at four centres out of five in 1934, and at six centres out of seven in 1935,

(ii) in no experiment in any year was the difference between the yields with early and late nitrogen significant,

(iii) the double dressing of nitrogen gave significantly higher yields than the single dressings at three centres in 1933, three in 1934, and five in 1935, and

(iv) in no case was the increase in yield with the double dressing over the control significantly greater than the sum of the increases with the early and late dressings, i.e. there is no evidence of a positive interaction between the early and late dressings which might occur if they acted in different ways.

The straw figures show that:

(i) a significant increase due to nitrogen occurred at all centres,

(ii) the early dressing gave a significantly greater yield than did the late dressing at two centres in 1933, at one centre in 1934 and at one centre in 1935,

(iii) the double dressing gave significantly greater yields than both single dressings at four centres in 1933, three in 1934 and six in 1935, and

(iv) the increase in yield with the double dressing over the control was significantly greater than the sum of the increases with the single dressings

Table III. *Details of spring top-dressing experiments at outside centres*

| Year | No. | Centre | Variety | Previous crop | Early N applied | Late N applied | Cut | Thrashed |
|---|---|---|---|---|---|---|---|---|
| 1933 | 1 | Quy, Cambs | Squarehead's Master | Wheat | 21 February | 26 April | 27 July | 18 August |
| | 2 | Good Easter, Essex | Squarehead's Master | Potatoes | 2 March | 25 April | 1 August | 16 August |
| | 3 | Whitchurch, Hants | Squarehead's Master | Wheat | 21 March | 30 May | 31 July | 14 August |
| | 4 | Thorpe Morieux, Suffolk | Yeoman | Oats | 1 March | 27 April | 2 August | 17 August |
| | 5 | Albury, Surrey | Squarehead's Master | Mangolds | 22 March | 29 May | 4 August | 15 August |
| | 6 | Kettering, Northants | Standard Red | Barley | 8 March | 13 May | 2 August | 22 August |
| | 7 | Friskney, Lincs | Little Joss | Wheat | 7 March | 28 April | 4 August | 23 August |
| | 8 | Knutsford, Cheshire | Standard Red | Potatoes | 28 March | 23 May | 8 August | 25 August |
| | 9 | Llangarron, Hereford | Standard Red | Ley | 14 March | 25 May | 3 August | 29 August |
| 1934 | 10 | Brackley,* Northants | White Victor | Wheat | 27 March | 23 May | 3 August | 27 August |
| | 11 | Saxondale, Notts | ? | Potatoes | 28 March | 17 May | 1 August | 3 September |
| | 12 | Potter Hanworth, Lincs | Little Joss | Oats | 28 March | 17 May | 8 August | 31 August |
| | 13 | Pickering, Yorks | Little Joss | Oats | 7 April | 15 May | 17 August | 5 September |
| | 14 | Stokesley, Yorks | White Wonder | Oats | 6 April | 16 May | 14 August | 6 September |
| 1935 | 15 | Winterbourne, Wilts | Cone | Wheat | 18 March | 13 May | 21 August | 6 September |
| | 16 | Heddington, Wilts | ? | Wheat | 18 March | 13 May | 22 August | 3 September |
| | 17 | Shifnal, Salop | Wilhelmina | Wheat | 22 March | 18 May | 6 August | 13 September |
| | 18 | Bickerstaffe, Lancs | Wilhelmina | Potatoes | 21 March | 27 May | 13 August | 17 September |
| | 19 | Orston, Notts | Swedish Iron | Tares | 22 March | 16 May | 5 August | 11 September |
| | 20 | Fencehouses, Durham | Wilhelmina | Oats | 28 March | 20 May | 14 August | 19 September |
| | 21 | Helperby, Yorks | Little Joss | Potatoes | 27 March | 22 May | 9 August | 18 September |

* ¾ cwt. sulphate of ammonia per acre applied to all plots in the autumn of 1933.

Table IV. *Results of spring top-dressing experiments at outside centres*

| | Centre no. | Grain, cwt. per acre: No N | N early | N late | N early and late | S.E. | Straw, cwt. per acre: No N | N early | N late | N early and late | S.E. | 1000 grain weight, g.: No N | N early | N late | N early and late |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1933 | 1 | 16·5 | 17·8 | 18·5 | 19·1 | 0·60 | 27·1 | 30·7 | 30·9 | 33·4 | 0·58 | 48·4 | 48·3 | 47·6 | 46·8 |
| | 2 | 29·5 | 30·4 | 30·3 | 31·2 | 0·63 | 52·1 | 55·4 | 57·3 | 57·7 | 1·76 | 49·1 | 48·0 | 48·5 | 47·9 |
| | 3 | 12·1 | 14·2 | 14·3 | 16·4 | 0·35 | 18·8 | 21·6 | 21·1 | 23·6 | 0·56 | 40·6 | 40·8 | 41·1 | 41·5 |
| | 4 | 17·2 | 19·6 | 20·6 | 21·9 | 0·44 | 30·4 | 35·2 | 38·2 | 43·6 | 1·11 | 38·5 | 37·6 | 37·9 | 38·1 |
| | 5 | 17·0 | 19·3 | 18·0 | 20·4 | 0·61 | 29·3 | 32·9 | 29·1 | 34·5 | 1·12 | 47·0 | 48·4 | 47·9 | 48·1 |
| | 6 | 15·8 | 18·9 | 18·3 | 21·7 | 0·66 | 25·2 | 30·4 | 26·3 | 31·4 | 0·86 | 45·9 | 45·7 | 45·3 | 45·0 |
| | 7 | 27·3 | 29·5 | 28·3 | 31·1 | 1·05 | 50·7 | 56·6 | 52·7 | 58·0 | 1·84 | 48·1 | 48·0 | 47·1 | 46·1 |
| | 8 | 34·4 | 36·9 | 37·9 | 38·8 | 1·07 | 51·3 | 56·1 | 53·2 | 56·1 | 2·00 | 42·4 | 43·1 | 41·8 | 39·7 |
| | 9 | 29·7 | 30·4 | 31·2 | 32·0 | 0·42 | 44·3 | 46·4 | 45·5 | 50·7 | 1·40 | 46·2 | 44·9 | 46·2 | 44·0 |
| | Mean | 22·2 | 24·1 | 24·2 | 25·8 | — | 36·6 | 40·6 | 39·4 | 43·2 | — | 45·1 | 45·0 | 44·8 | 44·1 |
| 1934 | 10 | 29·6 | 30·9 | 32·5 | 32·6 | 1·17 | 52·5 | 55·7 | 57·7 | 58·6 | 1·99 | 37·3 | 36·8 | 36·5 | 35·9 |
| | 11 | 30·5 | 31·2 | 30·9 | 32·8 | 0·65 | 55·7 | 58·8 | 57·5 | 61·4 | 1·54 | 46·5 | 46·6 | 46·4 | 45·9 |
| | 12 | 24·2 | 27·3 | 28·4 | 32·1 | 0·51 | 42·0 | 46·8 | 45·5 | 56·1 | 0·90 | 47·3 | 46·5 | 48·3 | 47·6 |
| | 13 | 22·4 | 27·0 | 26·7 | 31·3 | 0·77 | 36·8 | 44·8 | 42·0 | 52·5 | 1·16 | 48·5 | 48·2 | 48·9 | 48·2 |
| | 14 | 22·8 | 24·4 | 25·9 | 29·2 | 0·99 | 30·2 | 34·8 | 35·4 | 42·3 | 1·36 | 39·8 | 39·3 | 40·1 | 39·9 |
| | Mean | 25·9 | 28·1 | 28·9 | 31·6 | — | 43·4 | 48·2 | 47·0 | 54·2 | — | 43·9 | 43·5 | 44·0 | 43·5 |
| 1935 | 15 | 18·8 | 21·7 | 22·5 | 24·7 | 0·59 | 35·0 | 40·4 | 41·6 | 48·2 | 1·39 | 46·2 | 48·5 | 48·4 | 47·5 |
| | 16 | 11·5 | 13·7 | 14·7 | 17·5 | 0·36 | 24·6 | 28·0 | 29·5 | 35·0 | 0·98 | 39·9 | 40·3 | 41·2 | 42·1 |
| | 17 | 18·1 | 24·5 | 25·0 | 30·6 | 0·37 | 28·4 | 42·5 | 35·5 | 47·7 | 0·50 | 43·5 | 41·9 | 41·1 | 41·5 |
| | 18 | 25·7 | 26·3 | 26·4 | 28·6 | 0·74 | 46·1 | 49·3 | 45·4 | 57·7 | 2·48 | 38·8 | 37·8 | 34·8 | 34·5 |
| | 19 | 31·0 | 28·9 | 31·4 | 31·1 | 0·87 | 60·2 | 60·0 | 60·7 | 61·8 | 1·97 | 41·4 | 42·6 | 41·0 | 40·0 |
| | 20 | 32·7 | 35·7 | 35·5 | 38·4 | 1·39 | 51·1 | 56·4 | 54·3 | 62·1 | 1·98 | 41·1 | 40·9 | 39·9 | 39·2 |
| | 21 | 15·5 | 22·1 | 18·8 | 27·7 | 1·93 | 24·8 | 36·0 | 29·1 | 45·0 | 2·68 | 51·3 | 52·0 | 50·5 | 52·0 |
| | Mean | 21·9 | 24·7 | 24·9 | 28·3 | — | 38·6 | 44·6 | 42·3 | 51·1 | — | 43·2 | 43·4 | 42·4 | 42·4 |

at one centre in 1933 and one in 1934, i.e. there was a significant positive interaction between the early and late dressings at two centres.

In Exp. 17, measurements were taken of ear size, etc.; the results are given in Table V. Nitrogen had no effect on the number of ears, so that the increase in yield of grain was entirely due to increased ear size. As nitrogen caused a 5% reduction in 1000 grain weight, it is obvious that the effect of nitrogen in increasing yields was due to an increase in the number of grains per ear. The figures in Table V also show that the late dressing of nitrogen increased the height of straw much less than did the early dressing.

Table V. *Analysis of yield. Experiment* 17, *Shifnal*, 1935

| | Ear length cm. | Ears per yard row | 1000 grain weight g. | Height of straw in. |
|---|---|---|---|---|
| No nitrogen | 4·72 | 61·0 | 43·5 | 35·5 |
| Early N | 5·41 | 61·5 | 41·9 | 42·6 |
| Late N | 5·34 | 60·3 | 41·1 | 39·5 |
| Early N + late N | 5·92 | 60·8 | 41·5 | 45·7 |
| Standard error | 0·106 | — | — | 0·623 |

The average increases in yield for *all* the experiments (i.e. including those where the response to nitrogen was not significant) are given in Table VI. The average increases in yield of grain from the single applications of nitrogen (23·3 lb. nitrogen per acre) agree very well with the average for the Rothamsted experiments, viz. 2·61 cwt. grain per acre. The figures in Table VI show, however, that response to nitrogen was considerably greater when the wheat crop followed a straw crop than where it followed roots or ley. It is interesting to note that regardless of this there are no indications of falling off with double dressing, even with wheat after roots or ley.

Taking the results as a whole, there are some indications that the magnitude of the response to nitrogen in any one year was less the higher the control yield which, in turn, was affected by previous cropping and manuring, but owing to the large number of variable factors involved (climate, soil, variety, time of application, etc.) this relation is by no means constant. It is equally difficult to draw any conclusions regarding response of different varieties, because comparisons of varieties are not independent of differences between soils at the various centres. There is evidence, however, that Squarehead's Master responded less than did Wilhelmina and Little Joss (Table VII; results fo. double nitrogen application).

Table VI. *Average increases due to nitrogen*

| | | | Grain, cwt. per acre | | | | Straw, cwt. per acre | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Previous cropping | Year | *No. of* Exp. | Early | Late | Early *and* late | *Control* yield | Early | Late | Early *and* late | *Control* yield |
| Cereals | 1933 | 5 | 2·2 | 2·2 | 4·2 | 17·8 | 4·5 | 3·4 | 7·6 | 30·4 |
| | 1934 | 3 | 3·1 | 3·9 | 7·7 | 23·2 | 5·8 | 4·6 | 14·0 | 36·3 |
| | 1935 | 4 | 3·6 | 4·1 | 7·5 | 20·3 | 7·1 | 5·5 | 13·5 | 34·8 |
| | All years | 12 | 2·9 | 3·3 | 6·2 | 20·0 | 5·7 | 4·4 | 11·1 | 34·2 |
| Roots, ley, or autumn N | 1933 | 4 | 1·6 | 1·9 | 3·0 | 27·7 | 3·5 | 2·1 | 5·5 | 44·3 |
| | 1934 | 2 | 0·9 | 1·6 | 2·6 | 30·1 | 3·1 | 3·5 | 5·9 | 54·1 |
| | 1935 | 3 | 1·7 | 1·5 | 5·1 | 24·0 | 4·7 | 1·4 | 11·2 | 43·7 |
| | All years | 9 | 1·5 | 1·7 | 3·6 | 27·0 | 3·9 | 2·1 | 7·5 | 46·3 |
| *All experiments* | 1933 | 9 | 1·9 | 2·1 | 3·7 | 22·2 | 4·0 | 2·8 | 6·6 | 36·6 |
| | 1934 | 5 | 2·2 | 3·0 | 5·7 | 26·0 | 4·8 | 4·2 | 10·8 | 43·4 |
| | 1935 | 7 | 2·8 | 3·0 | 6·5 | 21·9 | 6·0 | 3·6 | 12·5 | 38·6 |
| | All years | 21 | 2·3 | 2·6 | 5·1 | 23·0 | 4·9 | 3·4 | 9·6 | 38·0 |

Table VII. *Average increases in yield due to* 46 *lb. nitrogen per acre*

| | *Following a corn crop* | | | | | *All experiments* | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | *Grain, cwt. per acre* | | *Straw, cwt. per acre* | | | *Grain, cwt. per acre* | | *Straw, cwt. per acre* | |
| Variety | No. of exps. | Control yield | Increase due to N | Control yield | Increase due to N | No. of exps. | Control yield | Increase due to N | Control yield | Increase due to N |
| Squarehead's Master | 3 | 14·8 | 3·9 | 17·0 | 5·8 | 7 | 22·5 | 3·5 | 35·4 | 5·6 |
| Little Joss | 3 | 24·6 | 6·9 | 43·2 | 12·4 | 4 | 22·4 | 8·2 | 38·6 | 13·6 |
| Wilhelmina | 2 | 25·4 | 9·1 | 39·7 | 15·2 | 3 | 25·5 | 7·0 | 41·8 | 14·0 |

AUTUMN APPLICATION OF NITROGEN

The results obtained at five centres are given in Table VIII. In the three experiments in the South of England, autumn nitrogen did not significantly affect grain yields, and it only increased the yield of straw significantly at one centre (Marlborough), but spring nitrogen had a very marked effect on yields of grain and straw. At these three centres the rainfall in December 1934 was extremely heavy (8–9 in.).

In the experiment at Newmarket there were, on an average, significant increases in yields of grain and straw due to nitrogen, but the differences between the spring and autumn dressings were not significant. At Darlington, however, the autumn dressing gave a significantly higher yield of grain than the spring dressing. At these two centres the winter rainfall was moderate (3–4 in. in December).

It may be concluded from the above experiments that except when winter rainfall is very heavy, and eight inches in one month in the south of England is extremely rare, application of part of the nitrogen in autumn is likely to be an advantage for wheat grown as a second straw crop.

Table VIII. *Autumn and spring application of nitrogen: outside experiments*, 1934–5

1 N = 1 cwt. sulphate of ammonia per acre

| Centre | | | | | | Grain, cwt. per acre | | | | | Straw, cwt. per acre | | | | | Rainfall 1934 (in.) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No. | Locality | Variety | Previous crop | Autumn N applied | Spring N applied | No N | 2 N autumn | 2 N spring | 1 N autumn 1 N spring | S.E. | No N | 2 N autumn | 2 N spring | 1 N autumn 1 N spring | S.E. | Oct. | Nov. | Dec. |
| 1 | Sherborne, Dorset | Victor | Wheat | 2 Oct. | 12 Apr. | 13·2 | 12·7 | 18·9 | 16·2 | 0·47 | 24·6 | 24·5 | 37·7 | 32·1 | 0·82 | 3·25 | 1·79 | 8·23 |
| 2 | Marlborough, Wilts | Cones | Oats | 17 Sept. | 12 Apr. | 17·5 | 18·2 | 25·3 | 22·2 | 0·40 | 46·6 | 50·0 | 58·4 | 54·1 | 0·35 | 2·13 | 1·55 | 8·56 |
| 3 | Linkenholt, Hants | Little Joss | Wheat | 3 Oct. | 13 Apr. | 10·0 | 11·6 | 14·3 | 13·6 | 0·85 | 18·4 | 21·3 | 32·0 | 28·4 | 2·09 | 1·60 | 2·52 | 8·99 |
| 4 | Newmarket, Cambs | Wilhelmina | Wheat | 5 Oct. | 16 Apr. | 20·7 | 26·3 | 23·5 | 24·3 | 1·93 | 31·4 | 43·2 | 39·8 | 39·5 | 3·19 | 1·99 | 1·83 | 3·44 |
| 5 | Darlington, Durham | Crown | Wheat | 13 Oct. | 28 Mar. | 17·9 | 26·6 | 21·2 | 24·7 | 0·83 | 25·9 | 34·3 | 33·4 | 34·1 | 1·86 | 2·47 | 1·65 | 4·28 |

### Summary and conclusions

The results of thirty-five field experiments to study the effect of nitrogenous fertilizers on the yield of wheat are preseuted.

Spring applications of nitrogen significantly increased yields of grain in twenty-five out of twenty-eight experiments. The yield of grain was increased to the same extent whether the nitrogen was applied early (February–March) or late (April–May). In one experiment at Jealott's Hill nitrogen applied as late as 30 May significantly increased the number of ears, but it seems probable that the increase in yield of grain due to spring application of nitrogen is usually due less to increase in number of ears than to increase in size of ears. This increase in size of ears was due to an increase in the number of grains in each ear and not to an increase in 1000 grain weight. The yields of straw were significantly increased by spring applications of nitrogen in all the experiments. In several of the experiments, the early spring application of nitrogen gave significantly greater yields of straw than the late application; the early application also increased the length of the straw much more than did the late application.

Owing to favourable weather, lodging occurred in only one experiment, and in this case the crop which received its nitrogen early was much more lodged than that which received its nitrogen late. From the smaller increase in yield and height of straw with the late applications, it may be argued that late applications result in less liability to lodging while giving as great an increase in yield of grain as early applications.

In eleven out of twenty-one experiments at outside centres, double dressings of nitrogen (23 lb. nitrogen per acre applied early and 23 lb. nitrogen per acre applied late) gave significantly greater increases in yield of grain than single dressings. The double dressing also gave greater increases in yield of straw than either the early or late dressings, and it may be presumed that liability to lodging was correspondingly increased.

The magnitude of the increase in yield due to nitrogen is dependent on the level of yield, previous cropping and manuring, etc. The double dressing of nitrogen (46 lb. nitrogen per acre) gave an average increase of 6·19 cwt. grain and 11·14 cwt. straw per acre when the crop was taken after a cereal crop, and 3·57 cwt. grain and 7·47 cwt. of straw when after roots, ley and fallow. There are no indications that the relative effects of early and late spring dressings on yields of grain were in any way affected by rainfall, provided that the application of the late dressing was not delayed too far into June.

In three experiments conducted in the South of England in 1934–5,

autumn nitrogen had no effect on yields, but the winter rainfall at these centres was abnormally high, about 9 in. falling in December alone. In one experiment in Cambridgeshire the increases in yield with autumn and spring nitrogen did not differ significantly, but in an experiment in Durham autumn nitrogen gave a significantly greater increase in yield than spring application. It would appear that where wheat is taken after a cereal crop, and except where the normal winter rainfall is very heavy, it is an advantage to apply part of the nitrogen in the autumn. Since autumn nitrogen increases yields by increasing the number of ears per plant, and since late spring nitrogen increases yields largely by increasing ear size, it would appear that late spring application of nitrogen might be particularly effective if some nitrogen is applied in the autumn.

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# A CRITICAL SURVEY OF INVESTIGATIONS ON THE "WILTING COEFFICIENT" OF SOILS

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From their investigations of the conditions under which plants exhibit "permanent wilting" Briggs & Shantz (1912) were led to propound certain generalizations which have since become subjects of acute controversy. Recent investigations have, however, made it possible to understand and explain many apparently contradictory experiments. The time is therefore ripe for reviewing the available information on the subject and endeavouring to sort out the facts definitely proved and explained.

(1) *Definition.* The "wilting coefficient" of a soil was defined by Briggs & Shantz (1912) as "the moisture content of the soil (expressed as a percentage of the dry weight) at the time when the leaves of the plants growing in that soil first reach a stage of wilting from which they cannot recover in an approximately saturated atmosphere without the addition of water to the soil". This condition is called "permanent wilting", and is considered to be the result of the forces opposing the further removal of soil moisture exceeding the osmotic force exerted by the cell contents of the plant. If the plants are removed from the saturated atmosphere, loss of water from the soil to the air continues slowly through the plant tissues and goes on even after the death of the plant. During the last stage the plant acts simply as a medium for the transference of water, and the final result is the same as if the air and soil were in direct contact.

(2) *Determination.* Several precautions are considered essential for accurate determination. The soil should be carefully mixed and be at a uniform moisture content when introduced into the pots. The pots should be impervious, and the soil surface sealed so that water can only escape by passing through the plant. Sudden fluctuations of temperature should be avoided so as to prevent distillation of moisture.

By definition the plants are considered permanently wilted when they cannot recover in a nearly saturated atmosphere unless water be added to the soil. However, plants having aerial water storage tissues, or thick heavy leaves, do not have a well-defined wilting point. Some determinations of the "wilting coefficient" were carried out by Briggs & Shantz with

such plants by using a very ingenious method known as the "balance method".

(3) *Briggs & Shantz's conclusions.* From the results of an extensive series of experiments, in which over twenty different types of soils and more than 100 species or varieties of wild and cultivated plants were used, and about 1300 determinations of the "wilting coefficient" made, Briggs & Shantz arrived at the following conclusions:

(*a*) The "wilting coefficient" is practically independent of the kind of plant used as an indicator, whatever its stage of development.

(*b*) It is not materially influenced by the dryness of the air, by moderate changes in the solar intensity, or by differences in the amount of soil moisture available during the period of growth.

(*c*) It is linearly related with several other soil constants from which it can therefore be indirectly computed.

The evidence upon which the last conclusion must be rejected will first be set out. The direct evidence regarding conclusion (*b*) will then be given, followed by the direct evidence connected with conclusion (*a*).

4. *The possibility of computation from other soil constants.* Believing the "wilting coefficient" to depend exclusively upon the soil characteristics, Briggs & Shantz drew the conclusion that it should be linearly related to the so-called physical constants of the soil. Accordingly they gave a set of formulae connecting the "wilting coefficient" with the moisture equivalent, hygroscopic coefficient, moisture-holding capacity and the mechanical composition of the soil.

As pointed out by Keen (1931), the equations given, being based on the assumption of a linear relationship between the constants involved, can only be taken as first approximations, and the valnes of the numerical coefficients refer to a limited range of soil types. This criticism is particularly true in relation to calculation from the mechanical composition. Such a method is liable to fail altogether for soils rich in organic matter or calcium carbonate, since neither of these soil materials is specifically determined. Work & Lewis (1934) found differences of the order of 30% between "wilting coefficient" and the values deduced from the mechanical analysis.

Estimations from the moisture-holding capacity determined by Hilgard's method (Briggs & Shantz, 1912) are no better. This is illustrated by the figures of Briggs & Shantz, since they show variations from 2·27 to 3·40 for the ratio

$$\frac{\text{moisture-holding capacity} - 21}{\text{"wilting coefficient"}}.$$

The figure $\pm 0{\cdot}021$ given as the "probable error" seems to show that the method is very accurate. One must realize, however, that for a soil having a moisture-holding capacity of 60% the calculated "wilting coefficient" is 13·75, but, as the true ratio may be 2·27 or 3·40, the actual "wilting coefficient" can be 17·2 or 11·5. Thus, for the example considered, the method could underestimate the actual figure by 22%, or else overestimate it by 19%.

Veihmeyer & Hendrickson (1934) have proved that the method cannot be used without introducing large errors, which are not eliminated by standardizing the degree of granulation as in the Keen-Raczkowsky (1921) technique.

As for the hygroscopic coefficient method, the average ratio found by Briggs & Shantz between the "wilting coefficient" and the hygroscopic coefficient is 0·68, and the probable error is 0·018. The extreme values, however, are 0·556 and 0·815, showing that for a soil having a hygroscopic coefficient of 6% the calculated "wilting coefficient" could be 16% lower or 19% higher than the actual value.

Hilgard's hygroscopic coefficient is not a true equilibrium point, but merely an empirical observation measuring the water taken up from an approximately saturated atmosphere in an arbitrarily chosen period of time (Puri, 1925). In more recent methods the hygroscopicity is determined in atmospheres of a definite relative humidity below saturation, in order to obtain a definite equilibrium moisture. Several humidities have been proposed: 99% ($H_2SO_4$ 2% by volume), (Robinson, 1922), 10% $H_2SO_4$ (Mitscherlich, 1923); while 50% relative humidity has been suggested by Puri *et al.* (1925) as that in which equilibrium can be more quickly reached.

Ten % $H_2SO_4$ was tried by Botelho da Costa (1933), and an average ratio of 2·1 found with the "wilting coefficient". However, as the ratio ranged from 1·7 to 2·4, no real improvement was obtained.

The question was more fully investigated by Veihmeyer & Hendrickson (1934), using several humidities (over distilled water, and 3·3, 10 and 44% $H_2SO_4$); they confirmed that the method can lead to serious errors in the estimation of the "wilting coefficient".

Calculation from the moisture equivalent, which was considered by Briggs & Shantz as a very reliable indirect means of determining the "wilting coefficient", has unfortunately not proved any better when tested with very different soil types. Veihmeyer & Hendrickson (1928) found ratios between the moisture equivalent and the "wilting coefficient" varying from 1·73 and 3·82, i.e. differing widely from the average value 1·84 given by Briggs & Shantz. In a more recent publication Veihmeyer

& Hendrickson (1930) mention a ratio of 1·39. Shaw & Swezey (1935) found the ratio 1·2 to hold for a number of Hawaiian soils, and Schofield & Botelho da Costa (1935) mentioned a ratio as big as 8.

It is therefore to be concluded that the "wilting coefficient", though roughly related to any measurement expressing the heaviness of the soil, cannot be calculated with reasonable accuracy for any of the formulae given by Briggs & Shantz.

(5) *Influence of the environment.* Attention must be drawn here to the difference between the "permanent wilting" of Briggs & Shantz and the loss of turgor that may occur whenever the rate of transpiration exceeds the rate of water absorption. This last condition is only "temporary", in the sense that recovery is possible by transferring the plants to less severe conditions of evaporation. In the case of permanently wilted plants turgor is not recovered even by exposure in a nearly saturated atmosphere unless water is added to the soil.

This fundamental difference has not always been appreciated in discussing the influence of the environment upon the "wilting coefficient". Thus Miller (1931), for instance, presents the experiments of Brown (1912) as supporting the view that the "wilting coefficient" depends upon the atmospheric conditions, although Brown dealt with temporary and not permanent wilting.

According to Briggs & Shantz, the influence of the atmospheric conditions upon the "wilting coefficient" should be negligible. This opinion was put to a severe test, first by Caldwell (1913) and afterwards by Shive & Livingston (1914) in the Desert Laboratory of Tucson, Arizona. General atmospheric conditions at Tucson are described as "extreme dryness of the air, almost constant wind, high solar intensity and long sustained high temperature". It was therefore easy to create a range of evaporating conditions much wider than that covered by the experiments of Briggs & Shantz.

Caldwell found that if wilting took place in a shaded room in an almost saturated atmosphere the value of the "wilting coefficient" approached the value calculated from the moisture holding capacity, using the formula of Briggs & Shantz. If, however, wilting took place in the open air, with or without shading, the calculated "wilting coefficient" was always lower than the actual value. Under conditions of maximum transpiration, the excess of the actual over the calculated values sometimes reached 30–40%. The plants used by Caldwell were *Zea mays* L., *Phaseolus vulgaris* L., *Xanthium commune* Britton, *Martynia louisiana* Mill., and *Physalis angulata* var. *Linkiana* Gray.

He concluded that permanent wilting appears to be a condition of

general plasmolysis[1] with accompanying cessation of certain of the protoplasmic activities.[2] This condition is characterized by a water content of the functioning foliage which is nearly constant for any species. The reduction of the water content to this point is the resultant of the action of transpiration versus root absorption; if aerial conditions permit these two processes to go on at approximately equal and moderate rates until the water supply fails at its source, there remains in the soil a quantity definitely related to the physical constants of the soil—the "wilting coefficient" of Briggs & Shantz. If transpiration be long maintained at a rate considerably exceeding absorption, permanent wilting is attained irrespective of the presence in the soil of more than the theoretical quantity of water which corresponds to the "wilting coefficient" of these authors. This excess of soil water is more or less related to the aerial conditions prevailing throughout the period of wilting and rises with increase in the transpiration rate.

All Caldwell's deductions are based on the variation of the ratio between the "wilting coefficient" and the values calculated from the moisture-holding capacity. We have seen, however, that this ratio varies from soil to soil. Therefore the better agreement found between the calculated and the actual values of the "wilting coefficient", when these were obtained in moist chambers, is just a matter of coincidence, so that the general theory advanced falls to the ground.

The influence of the environment does not appear so clearly in his experiments as it is generally believed. For instance, in Series II (*Zea mays*, third wilting in the open) the "wilting coefficient" seems to increase with the evaporating power of the air, but in Series III (*Zea mays*, third wilting in the open) the "wilting coefficient" actually decreases while the evaporating power of the air increases, in spite of the fact that this is on the whole higher than that in Series II. It seems, therefore, that even

[1] This use of the term plasmolysis is inexact (Maximov, 1929). "The early stages of wilting and plasmolysis are similar so long as the cell wall is even slightly distended by turgor pressure. After this the paths of wilting and plasmolysis diverge. In plasmolysis the still shrinking protoplasm separates from the cell wall, which has ceased to contract, the space between protoplasm and wall being filled with the external solution. In wilting the protoplasm cannot separate from the cell wall, as the place of the external plasmolysing solution is taken by air, which is unable, in gaseous condition, to pass through the wall. By virtue of the cohesion between the particles of water permeating protoplasm and cell wall alike, the contracting cell wall contents draw with them the wall, which thus becomes compressed. As the cell wall is only capable of slight compression, it becomes folded and wrinkled during wilting."

[2] When permanent wilting is reached in a moist chamber a general death of the root hairs is observed.

the extreme evaporating conditions prevailing in Tucson may have no marked influence upon the "wilting coefficient".

The influence of the environment was later reinvestigated by Shive & Livingston (1914), also in the Tucson Desert Laboratory. They tried to show that the soil moisture at permanent wilting is a logarithmic function of the evaporating power of the air. This conclusion, based on a selection of the experimental results, is not very convincing. In one case at least (Series V, *Zea mays*) no influence whatever is apparent. All the experiments show such large differences between replicates that their significance is very doubtful. Furthermore, Livingston & Koketsu (1920) seem to be inclined to rely on visual judgment to decide when permanent wilting is reached, which is bound to lead to serious errors, especially when working under conditions of strong evaporation. On the whole all that the experiments of Caldwell and Shive & Livingston definitely prove is that substantial differences may be observed between the "wilting coefficient" according to whether the experiment is carried out under extremely low or extremely high evaporation rates.

A very thorough investigation was carried out on the same subject by Veihmeyer & Hendrickson (1934) with sunflower seedlings. Working under conditions of evaporation described as covering "any evaporating condition likely to be obtained with plants growing in the field", they failed to find any tendency for the "wilting coefficient" to be influenced by the environment.

These results are seen not to be in contradiction with those of Caldwell, and Shive & Livingston when the atmometric data given by these different authors are comparable. Whereas the highest evaporation rate considered by Veihmeyer & Hendrickson was 2·8 c.c. per hour, Caldwell worked with very low evaporation rates (less than 0·3 c.c. per hr.) in moist chambers, or else under rates of more than 3 c.c. per hr. For evaporation rates lower than 2·8 c.c. per hr. the differences found by Livingston can hardly be considered as significant.

It may therefore be concluded that up to a certain limit of evaporation rate the environment does not affect the "wilting coefficient". According to Veihmeyer & Hendrickson this limit, at least for the sunflower, covers "any evaporating condition likely to be obtained with plants growing in the field". Under extreme conditions of evaporation the soil moisture content, when the leaves do not recover turgor by a 24 hr. exposure in a saturated atmosphere, may be significantly higher. It is possible, however, that when wilting is produced under these extreme conditions, a longer period of exposure would permit recovery. Very likely as a resnlt

of the very quick absorption the soil is dried down to the "wilting coefficient" in the zone immediately in contact with the root hairs. Absorption of the remaining available water would have to take place through the dried zone, which is bound to be a very slow process. It is therefore possible that the condition dealt with by Caldwell as well as by Livingston was not permanent wilting, since 24 hours' exposure was always used by the former, while the latter apparently relied mostly on visual judgment.

(6) *Influence of the plant.* While conclusion (*c*) must be discarded and conclusion (*b*) slightly altered, conclusion (*a*) has been fully confirmed. The available data show that all plants give practically the same "wilting coefficient" in the same soil. The slight differences observed bear no relation whatever to the drought resistance of the plants, and are as a rule explainable by differences in volume and ramification of the root system.

Confirmatory experiments were carried out by Capalungan & Murphy (1930), Parsons (1924), Wadsworth & Das (1930) and Veihmeyer & Hendrickson (1934). These last authors investigated the behaviour of twenty-five species, including cultivated field crops, fruit trees and common weeds. Wadsworth found that elongation of young sugar cane stops altogether when the moisture content of the soil is reduced to the "wilting coefficient" as determined with beans (*Tethonia speciosa*) or sunflower (*Helianthus annuus*). This supports the view that the "wilting coefficient" is the lower limit of the soil moisture content that allows plant growth.

Thus, the main facts concerning the "wilting coefficient" that can be considered as definitely ascertained can be expressed as follows:

(1) Unless under extreme conditions of evaporation, the "wilting coefficient" is not significantly affected by the environment.

(2) The "wilting coefficient" is practically independent of the plant used as an indicator.

(3) The "wilting coefficient" cannot be calculated with sufficient accuracy from any of the formulae proposed by Briggs & Shantz.

(1) and (2) taken in conjunction indicate that the "wilting coefficient" depends only upon soil characteristics, while (2) seems to be in contradiction with the fact that the osmotic pressure of the cell sap varies in the different plants (Crump, 1913). In the light of the experiments of Schofield & Botelho da Costa (1935), and Botelho da Costa (1938), this fact can be easily interpreted. They found values of the "suction" of the soil water at the "wilting coefficient" ranging from 10,000 to 20,000 cm. for non-saline soils or, using the logarithmic ***pF*** scale, from ***pF*** 4·0 to 4·3.

The average $pF$ 4·2 = 16,000 cm. of water = 16 atm. suction agrees well with the best determinations by vapour pressure (Thomas, 1921, 1924, 1928; Edlefsen, 1934) and seed absorption methods (Veihmeyer & Hendrickson, 1934). In other words, permanent wilting corresponds to a critical value of the $pF$. This definitely proves, from a different point of view, that the "wilting coefficient" is actually a characteristic of the soil. A determination of the moisture content corresponding to $pF$ 4·2 on the drying curve affords a very accurate estimation of the "wilting coefficient" (Schofield & Botelho da Costa, 1935; Botelho da Costa, 1938). The fact that the curves connecting $pF$ and moisture content vary in shape and slope (Schofield & Botelho da Costa, 1935) explains why no constant relation can obtain for all soils between the "wilting coefficient" and any other of the so-called soil constants.

Let us now consider the plant physiological argument regarding the osmotic pressure of the cell sap. It is still a matter of controversy whether permanent wilting corresponds to a condition of impossibility of water absorption or is merely a result of the rate of water loss exceeding for some time the rate of water intake. The experiments concerning the influence of the environment upon the "wilting coefficient" furnish some indirect evidence in this connexion.

If the wilting experiment is carried out under conditions of high relative humidity, as in the moist chambers used by Caldwell, Shive & Livingston, and Veihmeyer & Hendrickson, the first condition undoubtedly obtains. According to Caldwell the plants go on absorbing water very slowly until the roots die. Under conditions of higher evaporation the soil moisture residue, when plants wilt permanently, remains the same until a certain limit of the evaporating power of the air is reached. Contrary to what happens in the moist chamber experiments the root hairs do not die. If the root hairs are still alive and the soil moisture residue is the same as that in the moist chamber experiments, it follows that the permanently wilted condition is still the direct result of impossibility of water absorption, unless we admit that there is some difference not big enough to be measured in terms of moisture content.

This points to a condition either of equilibrium or of a gradient in favour of the soil. Let us now consider this interpretation in the light of our knowledge of the two forces that finally control the extraction of water from the soil by the plant: the suction pressure of the plant and the soil back-pull.

We have now very definite information as regards the last factor in

non-saline[1] soils (Botelho da Costa, 1938), but data concerning the suction pressure of plants,[2] though fairly abundant, are not so reliable. Thus, according to Maximov (1929) we have no exact method of determining active root pressure, and recent work by Ernest (1935) tends to discredit the plasmolitic methods of Ursprung (1935) so far considered as the most accurate means of measuring suction pressure. In view of all these uncertainties as to the magnitude of the forces that plants can exert for extracting water from the soil, it is difficult to reach definite conclusions. However, some approach to the truth can be obtained by considering the highest values of the suction pressure that have been measured, and by admitting that the plants considered are able to exert those pressures before wilting permanently.

It is known that the suction pressure of the cell depends principally on (i) the kind of plant, (ii) the portion of the plant, (iii) the habitat.

Different kinds of plants in the same habitat have different osmotic pressures (Harris, 1916; Maximov, 1929). The difference is especially marked between the hygrophytes and the mesophytes on the one hand and the xerophytes and halophytes on the other. Plants of the second two groups have normally much higher osmotic pressures (Maximov, 1929), reaching over one hundred atmospheres (Miller, 1931). In this discussion only the first two groups will be considered, since they include all the important cultivated species and because very few wilting experiments have been carried out with xerophytic and none with halophytic species.

As a rule higher values have been measured in the leaves than in the roots (Dixon & Atkins, 1913, 1915, 1916; Hannig, 1912). The highest values measured in roots of hygrophytes and mesophytes vary round about 10–20 atm., whereas values of 30 or more atmospheres have been measured in the leaves (Dixon & Atkins, 1910). It is generally believed that as a rule a gradient in favour of the leaves is maintained (Shull, 1930).

The suction pressure is mostly regulated by the habitat. According to Miller (1931), under the same conditions specific differences are comparatively small. The xerophytes and halophytes should however be considered apart, since they can normally develop much higher pressures than the

[1] This designation as used here applies to soils having less than about 500 p.p.m. of soluble salts as deduced from conductivity measurements.

[2] Maximov's nomenclature (1929) of the osmotic quantities of the plant cell is used here. He limits the term suction pressure $S$ of the cell to the sense $S = P - T$, where $P$ is the osmotic pressure of the cell sap and $T$ the pressure of the cell wall on its contents.

other plants (Maximov, 1929). The osmotic pressure of the root hairs increases with the concentration of the external medium (Eaton, 1927; Hill, 1908) tending to maintain a gradient in favour of the plant (Roberts, 1916). Similarly, when the moisture content of the soil decreases the plant responds to the increasing *pF* of the soil by a rise in the osmotic pressure of the cell sap, and therefore of the suction pressure of the cell (Hibbard & Harrington, 1916; Drabble & Drabble, 1907; Molz, 1926).

The experiments of McCool & Millar (1917) are particularly interesting in this connexion. They determined by freezing-point the variation of the osmotic pressure of the cell sap in the roots and the tops of corn plants while the moisture content of the soil was reduced. The freezing-point of the soil was also measured. While the plants showed excellent or good condition the freezing-point was higher in the leaves than in the roots, and in these higher than in the soil. When wilting set in the freezing-point in the roots and tops was nearly equal, and lower than in the soil.

Since the suction pressure differs in the roots and the leaves, the question arises as to the organ that should be considered for comparison with the soil suction. Shull (1916, 1924, 1930), who first attempted to compare plant suction with the soil back-pull at the "wilting coefficient", considered only the suction pressure of the roots. But active root suction is not the only means by which plants absorb water from the soil. Though there is no complete agreement as to the relative importance of active and passive root suction, there is no doubt that a considerable portion of the water absorbed by the plants is sucked in passively by the roots under the influence of the strong suction transmitted to the roots from the transpiring leaves (Maximov, 1929). Usually during the night active suction prevails, while in the daytime, owing to a sharp increase of the suction pressure of the leaves, there is a prevalence of passive suction.

The maximum values found for the suction pressure of the roots of the hygrophytes and mesophytes vary as a rule from 10 to 20 atm. Taking these values as representing the maximum suction that these plants can exert to extract water from the soil, permanent wilting could be interpreted as a condition of equilibrium between the soil and the plant, since we find that 10–20 atm. are the limits of the soil suction at the "wilting coefficient" in non-saline soils.

If, however, we consider the suction pressure of the leaves, it seems that permanent wilting occurs in spite of a gradient in favour of the plant. In fact osmotic pressures of 30 and even 40 atm. have been measured in the leaves, and the actual suction pressure of the wilted cell is probably higher than the osmotic pressure of its contents when turgid (Parsons,

1924). Permanent wilting would therefore be, not a result of lack of water, but a consequence of a rate of absorption smaller than the rate of transpiration. Permanent wilting, however, is defined (p. 630) as a condition from which the plants do not recover in a saturated atmosphere. If passive absorption is possible in the advanced stages of wilting it is hardly understandable why there is no recovery in a saturated atmosphere, where transpiration is reduced to a minimum, if there is a gradient in favour of the plant.

The experiments of Bakke (1918) on the rate of transpiration afford a better interpretation of the phenomenon. It has been observed (Miller, 1931) that the transpiration rate falls suddenly with wilting but rises thereafter to a considerable extent subsequent to a fall once more as desiccation occurs. This initial fall in the transpiration rate represents the increase in incipient drying within the leaves. During this initial decrease in transpiration the water columns in the conducting tissues remain intact. As the drying of the leaves continues, the strain on the columns of water increases so that the breaking point is eventually reached. When the water columns break the tension is relieved and the transpiration rate is accelerated. When the water of the broken columns has been evaporated, the rate of transpiration will fall again. Bakke found good agreement between the "wilting coefficient" and the moisture content of the soil when the rate of transpiration indicated that the water columns of the stem had been ruptured (Bakke, 1918). If this be true, the suction pressure of the leaves at permanent wilting cannot be transmitted to the roots. Therefore recovery of turgidity will depend upon active root absorption.[1] As the maximum values of suction pressure in roots fall between 10 and 20 atm. (also the limits of variation of the soil suction at the "wilting coefficient") permanent wilting should correspond to a condition of equilibrium between active root suction and soil back-pull.

On the other hand, the steepness of the *pF* curves in the neighbourhood of the "wilting coefficient" shows that below this critical moisture content the back-pull of the soil increases so rapidly that it will quickly exceed the suction force of the plant. Either active or passive root absorption becomes therefore undoubtedly impossible a little below the "wilting coefficient". Nevertheless, if the plants are removed from the saturated atmosphere, further reduction of the soil moisture content is still possible by evapora-

[1] This view is supported by the fact that, according to Maximov (1929), wilted plants recover more rapidly when their roots are watered than when their shoots are cut off and placed in water.

tion through the plant tissues. This, as already noted, is an extremely slow process which proceeds even after the death of the plant.

It remains to be explained why all plants give practically the same "wilting coefficient" in the same soil, when it is known they differ as to the maximum suction they can exert. Differences of 5 or 10 atm. seem enormous from the physiologist's point of view, and seem to indicate—against the experimental evidence—that the "wilting coefficient" ought to be different according to the plant used as an indicator. Why it is not so is quite clear in view of the steepness of the *pF* curves in the neighbourhood of the "wilting coefficient". A difference of several atmospheres in suction corresponds actually to a very slight difference in the moisture content of the soil. Therefore the fact that all plants give practically the same "wilting coefficient" in each soil merely shows that the differences measured *in terms of moisture content* do not exceed the experimental errors.

*Saline soils.* The existing evidence shows that in the case of saline soils plants are able to overcome a higher *pF* of the soil before wilting permanently. Kearny (1913) found that the presence of an excess of soluble salts did not affect the ability of young wheat-plants to reduce ultimately the water content of the soil to the "wilting coefficient", unless the quantity of the salts was sufficient to induce marked pathological symptoms. Botelho da Costa (1938), on the other hand, found a higher *pF* at the "wilting coefficient" in the soils having more than about 600 p.p.m. of soluble salts. As the power of adaptation to soil salinity differs with the plant, it is reasonable to expect some discrepancies in the "wilting coefficient" of saline soils if different plants are used as indicators. Halophytic species would probably lead to smaller values than the other species.

## SUMMARY

The experiments of Briggs & Shantz led them to conclude that the "wilting coefficient" is a soil "constant" which is (*a*) independent of the kind of plant used as indicator, (*b*) independent of the conditions under which the plant was grown, and (*c*) directly related to several other soil constants.

Subsequent research as well as an examination of their own results has shown that (*c*) is untrue, while (*a*) and (*b*) are substantially correct for hygrophytes and mesophytes. Earlier writers have been led to wrong conclusions regarding (*a*) and (*b*) through assuming (*c*) to be correct and

through disregarding the particular nature of "permanent wilting" as defined by Briggs & Shantz.

The fact that considerable variation is to be found between the osmotic pressure found in different plants, in different parts of the same plant and in the same part under different conditions is not at variance with conclusions (*a*) and (*b*) when properly understood.

An important factor making for the substantial constancy of the "wilting coefficient" for a given soil is the extreme steepness of the curve connecting suction pressure and soil moisture content, in consequence of which differences of suction pressure of unquestionable significance from the standpoint of plant physiology give rise to differences in soil moisture content that are too small to be detected.

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# THE MEASUREMENT OF *pF* IN SOIL BY FREEZING-POINT

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(With One Text-figure)

Bouyoucos & McCool (1915) undertook freezing-point determinations as a means of measuring the actual concentration of the soil solution directly in the soil at different moisture contents. Only with sand, however, *did they find the depression of the freezing-point to be nearly inversely* proportional to the moisture content. In general the freezing-point depression increases more nearly in geometrical progression as the water content decreases in arithmetical progression. They nevertheless adhered to the view that the depression was a reflexion of the salt concentration, and advanced the hypothesis that part of the water that is driven off when the soil is oven-dried is "unfree", i.e. not available for the solution of salts. Keen (1919) criticized their interpretation, showing that, according to their definition, the amount of "unfree" water must vary with the total moisture content. This theory did not enable them to account for a close similarity which they observed between the lowest moisture content at which they were able to induce freezing (depression just over 1° C.) and the "wilting coefficient" of Briggs & Shantz, and they at first thought it a coincidence. Subsequently (1916) they concluded that there must be some fundamental connexion here, a view that Bouyoucos has recently (1936) reaffirmed.

Parker (1921 *a*, 1921 *b*, 1922) later showed that large depressions can be obtained in soils and other granular materials washed free of all soluble material, and that the effect of dissolved material when present is superimposed on that of the finely divided insoluble material, the two effects being very nearly additive. When both are present, the characteristic of the contribution of the solid matter is, in general, a change from a very small depression, say 0·01° C. to a relatively large one, say 1° C., for perhaps a threefold reduction in moisture content which might change

the contribution from dissolved substances from, say, 0·04–0·12° C. In such a case the effect of the soluble matter is predominant in the wetter condition but the reverse is true in the drier condition. Thus it was possible for Hoagland (1918), by using rather high moisture contents, at which the solids make a very small contribution to the freezing-point depression, to use this measurement to follow changes in the concentration of the soil solution. On the other hand, when soils containing less than about 500 parts per million of salts have their moisture content reduced far enough for the freezing-point depression to exceed about 0·3° C., this depression is mainly due to the development of a pressure deficiency or suction in the water that remains. The equation

$$H = \frac{Lj}{Tg} t$$

has been used by Schofield (1935) to compute $H$, the lowering of the free energy, from $t$, the depression of the freezing-point. $H$ is expressed as the height in centimetres of a column of water and, in the absence of salts, is equal to the suction. This equation rests on the assumption that the ice with which the water under suction establishes equilibrium is in the form of relatively large crystals under atmospheric pressure. A direct experimental test was made by packing about 20 g. of moist salt-free soil in a boiling tube as for a freezing-point determination and inserting in the hole, afterwards to be occupied by the bulb of the Beckmann thermometer, a very small filter candle attached to a suction pump. The tube was held upside down so that any water extracted from the soil would run down into a side-tube. After one or two trials the moisture content was found which just gave a measurable discharge (about 0·1 c.c.). The filter was then extracted and the freezing-point determined. Since $H$ had been adjusted to one atmosphere (the full suction of the filter pump) $= 10^3$ cm., the freezing-point depression should have been 0·083° C. This was found to be the case within experimental error for a number of clays, but there was a notable and consistent departure in the case of a soil from Rothamsted Park Grass. This soil suffers a considerable reduction in moisture content for quite a small increase in applied suction when this is of the order of $10^3$ cm. and must therefore contain a lot of pores with a mean diameter at their opening of about $4\sigma/10^3$ g. $= 3 \times 10^{-4}$ cm.

Although the depressions of about 0·04° C. observed with the Park Grass soil were reproducible to at least 0·01° C., and ppeared, therefore, to be significantly less than the theoretical value 0·083, we cannot feel quite as confident about this result as we do about the much larger

depressions discussed in the latter sections of the paper. More observations on this point are desirable.

Provided that the departure from 0·083° C. is real, and not due to imperfections in technique, it appears that ice is able to invade pores of this size and so come under the influence of the suction of the water in them.

For the same complication to arise when $H$ is about $10^4$ cm., ice would have to be able to invade pores with openings only $3 \times 10^{-5}$ cm. across, for, regardless of the texture of the material, only pores smaller than these could remain full of water. This it does not appear able to do. The fact, foreshadowed by Bouyoucos & McCool and recently established by Schofield & Botelho da Costa (1935), that in non-saline soils the moisture content at which a soil gives a freezing-point depression of 1·2° C. is within experimental error of the "wilting coefficient" is rationally accounted for if in both tests the same thing is being measured, namely a critical suction. From equation (1) we find this suction to be about $1{\cdot}6 \times 10^4$ cm. If there were any influence of texture such as is possible at $10^3$ cm. the freezing-point depression at the "wilting coefficient" should be lower in light soils, but no such effect is manifest. Moreover, all the estimates that can be made from other data confirm the value obtained from freezing-point. It appears, therefore, that equation (1) may be used with complete confidence for calculating the free energy lowering, $H$, from freezing-point depressions above about 0·3° C. On account of the rapid increase of freezing-point depression with falling moisture content it is convenient to work with the logarithm of the free energy lowering for which the symbol $pF$ has been proposed. Thus, substituting numerical values we have

$$pF = \log_{10} H = 4{\cdot}1 + \log_{10} t.$$

The particular value of freezing-point determinations is that they bridge the gap[1] between $pF$ 4·8, the lowest value readily controllable by vapour pressure, and $pF$ 3, the greatest that can be applied through a filter attached to a vacuum pump. The fact that in certain cases the freezing-point equation may become unreliable below $pF$ 3·5, and cannot be carried for experimental reasons above $pF$ 4·4, does little to reduce its utility, since it can be applied just where it is most wanted.

[1] This statement only applies to water, the gap is less for liquids with higher molecular weights.

### General considerations

The determination of the freezing-point of a sample of moist soil is rendered possible by the fact that it can be supercooled if left undisturbed in a freezing mixture, and that freezing can be induced by jarring. Bouyoucos & McCool recommend a supercooling of about 1° C. below the expected freezing-point, on the ground that this gives the most rapid freezing. Once it is appreciated that freezing *dries* the soil sample there is a further reason for carefully controlling the supercooling. The freezing temperature observed is not that of a sample having the original moisture content (or that after thawing) but of one having a moisture content less by the amount of ice formed. It is of little use to make a highly accurate observation of the temperature unless corresponding care is taken to find the true value of the moisture content to which it corresponds. The taking of additional precautions is, however, time-consuming. Consequently two procedures have been developed. Procedure A embodies all precautions which it appears desirable to take in the interests of accuracy. Procedure B is less elaborate and sufficiently accurate for certain purposes such as the indirect determination of the wilting coefficient.

The apparatus used only differs from that described by Bouyoucos & McCool in the use of a wide-mouthed vacuum flask to hold the freezing mixture and of a tapper made from an electric bell operating on the stem of the Beckmann thermometer. The temperatures could be more exactly read on an electrical thermometer, but, as the accuracy of the determinations is principally limited by uncertainty as to the true moisture content and the difficulty of precisely controlling the conditions, the purchase of a more expensive instrument did not seem justifiable.

*The Beckmann thermometer.* If, after some weeks' storage at room temperature, a Beckmann thermometer is used to determine the freezing-point of pure water and the determination is immediately repeated several times, it will give successively higher readings which only agree within experimental error after half a dozen or more repeats. If, however, the thermometer, when not in use, is kept with its bulb in ice and water the effect is greatly reduced and the second determination in pure water usually checks with the first to within two or three thousandths of a degree. It is necessary, however, to redetermine the zero in this way each morning and afternoon that the thermometer is in use.

*Experimental procedure.* The practical difference between procedures

A and B is that in A three freezing mixtures are used, in B only one. The extra freezing mixtures are used in A in order

(1) to give a more delicate control of the degree of supercooling;

(2) to reduce as far as is practicable the cooling due to the surroundings while freezing is in progress.

In both cases the boiling tubes containing the soil samples to be tested are placed in an ice-pail until needed. The Beckmann thermometer is then inserted in the tube selected for the first determination so that the soil is packed snugly round the bulb, and the tube is then dipped into a cooling mixture at about $-3°$ C. until the temperature has been depressed below $0°$ C. by about half the amount desired. The tube is then removed, rapidly wiped, and inserted in the wider tube so that it is now surrounded by an air jacket. Thus partially insulated it is further slowly cooled to desired temperature, which is usually about $1°$ C. below the expected freezing-point.

In procedure B, which is substantially that used by Bouyoucos & McCool, this further cooling is carried out with the air jacket surrounded by the freezing mixture at $-3°$ C., and as soon as the desired temperature is reached freezing is started by giving a sharp twist to the Beckmann, the automatic tapper is set going, the maximum temperature is recorded and all that remains to be done is to determine the moisture content of the soil sample in the ordinary way.

In procedure A the further cooling is done in a second freezing mixture adjusted to the temperature required for supercooling, and the sample is kept there until there is no further change in temperature during several minutes. Here the freezing mixture at $-3°$ C. is only used to save time. The initial cooling can be carried nearly to the point finally desired, but in this case great care is needed in wiping the soil tube and inserting it into the wider one, as a slight jolt may start freezing and spoil the run. The steady temperature is noted and the freezing is started. When the temperature has risen to within about half a degree of the expected freezing-point, the soil tube, still surrounded by the outer tube, is rapidly transferred to a third freezing mixture at this temperature. The tapper is then started and the maximum reading of the Beckmann recorded.

*Calculation of the amount of ice formed.* With both procedures a minimum estimate can be made of the ice formed, if the water equivalent of the tube containing the soil and thermometer bulb is known. This can be found quite simply by cooling it to $0°$ C. and then immersing it in some water at about room temperature contained in a vacuum flask and noting the fall in temperature. With the tube and thermometer used by us,

containing soil at 15% moisture content weighing 20 g. when dry, 300 g. of water had its temperature reduced from 20·700 to 19·895:

$$\text{Water equivalent} = \frac{300\ (20{\cdot}700 - 19{\cdot}895)}{19{\cdot}895} = 12{\cdot}1.$$

The average for a number of experiments gave 12·0. As 3 g. of water were present the water equivalent of the solids was 9 g.

In the general case where the water equivalent of the solids is $W$ g., the weight of soil (when dry) $S$ g. and the moisture present is $M$ g. % of dry soil, the heat derived from the formation of ice per 1° C. rise in temperature during freezing is

$$\left(W + \frac{MS}{100}\right) \text{ calories.}$$

Taking 80 calories per gram as the latent heat, the ice formed is

$$\frac{1}{80}\left(W + \frac{MS}{100}\right) \text{ gram.}$$

This, expressed as a percentage of the dry weight of soil, is

$$\frac{1}{80}\left(W + \frac{MS}{100}\right)\frac{100}{S} = \frac{W}{80} \cdot \frac{100}{S} + \frac{M}{80}.$$

In the particular case considered above, $W = 9$ and $S = 20$, so that the reduction in moisture percentage caused by ice formation is

$$(0{\cdot}56 + 0{\cdot}0125\ M) \times C,$$

where $C$ is the rise in temperature during freezing.

In the case of procedure A this calculation should not fall far short of the amount actually formed, because precautions are taken to reduce as far as practicable the further formation of ice due to continued cooling by the surroundings while freezing is in progress. To test the correction formula, five soils of different textures each at several moisture contents were tested by procedure A using two degrees of supercooling (about 1 and 2° C. below the freezing-point). Expressed as $pF$, the observed freezing-point depressions are plotted against both the corrected and uncorrected moisture contents in the accompanying figure.

The alignment of the corrected points is very satisfactory. It serves not only to check the correction formula but also to provide a striking confirmation of the view that freezing *dries* the soil, upon the truth of which, as we have already emphasized, the validity of the equation for calculating $pF$ from freezing-point depression ultimately rests. The test was thus a crucial one.

*Effect of further cooling during freezing.* Although the additional precautions introduced into procedure A greatly reduce cooling by the surroundings subsequent to the starting of freezing, this is not altogether prevented. The further cooling scarcely depends on the degree of supercooling, so that the test described above is neither influenced by it nor throws any light upon it. Some idea of the magnitude of the effect may, however, be obtained by comparing the results obtained by procedures A

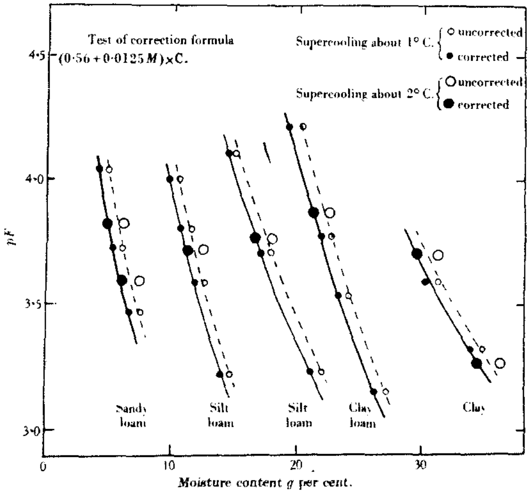


Fig. 1.

and B. Consider a typical case in which the observed depression is 0·5° C. During the greater part of the time that elapses between the starting of freezing and the recording of the maximum temperature the soil sample is nearly 2·5° C. above the surroundings in the case of procedure B, but never as much as 0·5° C. in the procedure A. If the same time were required to reach the maximum temperature the further cooling would lead to the formation of five times more ice in procedure B than in procedure A. The ratio is somewhat reduced by the fact that the

greater cooling in B will cause the temperature to reach its maximum sooner, but we will not be far out in estimating that four times as much additional ice is formed in B as in A. Hence for a recorded freezing-point depression of 0·5° C. the difference between the additional ice formed in A and B is in this case about three times the error of A and three-quarters of the error of B. The line in Table I corresponding to 0·5° C. depression (*pF* 3·8) gives an average difference of 0·5% and therefore suggests that the error in A is of the order of 0·2% and in B of 0·7%.

Table I

| *pF* | Difference in moisture percentage: Columbia sand | Columbia silt loam | Farwell silt loam | Aiken clay loam | Stockton clay adobe |
|---|---|---|---|---|---|
| 4·2 | 0·22 | 0·25 | 0·32 | 0·30 | 0·36 |
| 4·1 | 0·22 | 0·25 | 0·45 | 0·31 | 0·49 |
| 4·0 | 0·27 | 0·40 | 0·60 | 0·35 | 0·50 |
| 3·9 | 0·21 | 0·40 | 0·65 | 0·37 | 0·55 |
| 3·8 | 0·24 | 0·49 | 0·68 | 0·38 | 0·70 |
| 3·7 | 0·30 | 0·60 | 0·74 | 0·45 | 0·78 |
| 3·6 | 0·30 | 0·63 | 0·90 | 0·50 | 0·90 |
| 3·5 | 0·44 | 0·75 | 0·94 | 0·50 | 1·20 |
| 3·4 | 0·46 | 0·75 | 1·05 | 0·60 | — |
| 3·3 | — | 0·90 | 1·13 | 0·80 | — |

In the case of procedure A the further cooling only differs from case to case owing to the different times taken to reach the maximum temperature. It seems probable, therefore, that a further term could be added to the correction formulae, namely a constant multiplied by the product of the time in minutes during which the soil is in the last freezing mixture before the maximum temperature is reached and the maximum temperature difference between the soil and surroundings. We cannot say more here than to suggest that the constant might be about 0·2. The correction in this case is small, and other conditions would have to be very carefully regulated before any useful purpose would be served by applying it.

With procedure B the case is rather different. Since several times as much ice is formed by further cooling a correction should be made, but this is difficult to do because the difference in temperature between the soil and the surroundings depends on the freezing-point.

In view of the empirical fact that the curve connecting *pF* and moisture content, as found by freezing-point measurements, is steeper for high freezing-point depressions than for low, and also tends to be steeper for light soils (which reach the maximum temperature sooner) than for heavy soils, it turns out that the curve derived by procedure B can be more nearly made to coincide with that for procedure A by a bodily shift in the

direction of the $pF$ axis than by a shift in the direction of the moisture content axis. The average shift needed is about 0·05 $pF$ unit. Thus a determination, accurate enough for many purposes, may be made by procedure B of the true relationship between $pF$ and moisture content by subtracting from the observed moisture content the correction given in the last section and also subtracting 0·05 from the $pF$ calculated from the observed freezing-point depression. It should be realized that the subtraction of 0·05 (which is equivalent to decreasing the observed freezing-point depression by 11 %) is an empirical device and must not be taken to imply that the observed freezing-point depression is incorrect. Provided equilibrium has been established, the depression recorded by the thermometer is the true one for the soil sample in moisture condition in which it exists at that time. The difficulty, more particularly when procedure B is used, is to know exactly what is that moisture condition.

*Successive freezings.* Bouyoucos & McCool, using procedure B, generally found that a second experiment on the same tube of soil gave a smaller freezing-point depression than the first. This observation has been confirmed and a clue obtained as to the reason. Since it has been observed that a longer time is needed to reach the maximum temperature in the first freezing, we are naturally led to the view that the difference arises from the greater further cooling in this case. This idea is supported by the observation that the difference is much less when procedure A is used, which in turn provides additional evidence that further cooling in procedure A produces only a minor disturbance.

It is, generally speaking, with the finest grained materials that the longest time elapses after freezing has been started before the maximum temperature is reached. This is in accord with the view that in these cases water has to *move* through the finer pores to build ice crystals in a relatively small number of large cavities. On the first freezing, if the cavities are at first too small to accommodate the ice, the crystals as they grow may enlarge them by pressing aside the plastic mass. These cavities will not return to their original size on thawing so the formation of ice on the second and subsequent freezing will be easier and quicker.

*Preparation of the soil.* A detailed discussion of the way in which the previous history of the soil affects the relationship between the freezing-point depression and the moisture content is outside the scope of this paper. It must, however, be pointed out that the degree of reproducibility that is seen in the foregoing curves is only obtained when care is taken to standardize the method of incorporation of the water with the air-dry soil and of packing it in the tube, and when a fixed time (say

48 hr.) elapses between the wetting of the soil and the taking of the freezing-point.


## Summary

Two procedures are described for ascertaining the relationship between the freezing-point and moisture content of a soil. Since the soil sample is dried in the process of freezing, it is necessary to estimate how much water has been frozen out of the soil at the moment when the freezing temperature is recorded.

Procedure A embodies all the precautions which appear desirable when the greatest accuracy is required. Procedure B is simple and rapid and yet accurate enough for routine estimations.


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# THE INDIRECT DETERMINATION OF THE "WILTING COEFFICIENT" BY THE FREEZING-POINT METHOD, AND THE INFLUENCE OF THE SALTS UPON THE *pF* AT THAT CRITICAL MOISTURE CONTENT

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(With Two Text-figures)

The term "wilting coefficient" was proposed by Briggs & Shantz (1912) to designate the percentage of water at which in a given soil the plants wilt permanently. This paper deals with the problem of determining this critical moisture content indirectly, i.e. by dispensing with the laborious and time-consuming wilting test. Other designations have been suggested by Veihmeyer & Hendrickson (1934) to distinguish between the values obtained by wilting experiments and those determined indirectly. In the writer's opinion, however, it is preferable to keep the original designation with its original meaning. Therefore, in what follows, the designation "wilting coefficient" refers to the values obtained directly by wilting tests, and the words "indirectly determined" will be added when necessary. All possible confusion is thus avoided without introducing new terms that would prove misleading.

The first attempt at indirect determination was made by Briggs & Shantz (1912), who tried to relate the "wilting coefficient" to other soil physical constants, namely the moisture equivalent, the hygroscopic coefficient, the moisture-holding capacity and the mechanical composition of the soil. The validity of the formulae given by these authors has been discussed in detail in another paper by Botelho da Costa (1938). It is sufficient to state here that it has been quite definitely established that the "wilting coefficient" cannot be computed with any reasonable accuracy by any of them.

With the exception of the mechanical composition, the other soil constants mentioned can be defined as "the moisture percentages that a soil retains or absorbs under some definite set of conditions". These

moisture percentages are either lower (hygroscopic coefficient) or higher (moisture equivalent, moisture-holding capacity) than the "wilting coefficient". The possibility of indirect determination seems to lie with some method that, at least to some extent, copies the action of the plant in drying the soil down to a moisture percentage equal to the "wilting coefficient". Bouyoucos & McCool (1915), while attempting to use freezing-point depression to determine the concentration of the soil solution, chanced on the fact that the lowest moisture content at which freezing can be induced is close to the "wilting coefficient". The realization by Schofield (1935) that freezing *dries* the soil provided the necessary clue. The first step in this direction was to ascertain the depression of the freezing-point at the "wilting coefficient". This was done by Schofield & Botelho da Costa (1935), who used the freezing-point method[1] as a means of measuring the $pF$[2] of the water in soil. The $pF$ on the drying curve was measured in a set of Portuguese soils between the moisture equivalent and the "wilting coefficient". The values obtained at these two moisture contents are given in Table I.

Table I

| Soil | Wilting coefficient | $pF$ at the wilting coefficient | Moisture equivalent | $pF$ at the moisture equivalent |
|---|---|---|---|---|
| Colares dune sand | 0·50 | 4·24 | 1·18 | — |
| Niza sandy | 2·88 | 4·04 | 11·51 | 2·70* |
| Belas sandy | 3·68 | 4·23* | 12·16 | 2·69* |
| Castelo Branco sandy loam | 4·60 | 4·16 | 15·11 | 2·51* |
| Colares calcareous | 3·09 | 4·35 | 24·28 | 2·70* |
| Lisboa clay | 11·63 | 4·40* | 28·19 | 2·82 |
| Alges clay | 13·37 | 4·40* | 29·30 | 2·94 |
| Oeiras clay loam | 21·55 | 4·09 | 31·40 | 2·96 |
| Average | | 4·24 | | 2·76 |

* Obtained by extrapolation.

The values measured at the moisture equivalent agree reasonably well with the theoretical figure 2·9. It was pointed out, however, that a truly constant figure could not be expected, especially if soluble salts be present.

At the "wilting coefficient" the $pF$ varies from 4·0 to 4·4, though the "wilting coefficients" range from 0·5 to 21·6 %. The variation does not bear any relation to the soil texture, and was suspected to be partly due to insufficient care in sampling. The average value (4·2) was found to agree well with the vapour pressure determinations carried out by Thomas

[1] The technique used is described as "procedure A" by Schofield & Botelho da Costa (1938).

[2] The $pF$ is the positive logarithm of the height in centimetres of a water column that is equivalent to the free energy depression of the water in the soil (Schofield, 1935).

(1921, 1924, 1928) and Edlefsen (1934) and the seed absorption measurements of Veihmeyer & Hendrickson (1934). 4·2 was accordingly taken as the critical *pF* of the soil at which plants wilt permanently. The differences found in the shape and slope of the *pF* curves showed that no constant ratio can be expected to hold for all soils between the moisture contents corresponding to any two different values of the *pF*, such as the moisture equivalent (*pF* 2·9) and the "wilting coefficient" (*pF* 4·2). Therefore a determination of the moisture content giving a *pF* of 4·2 in the drying curve is the only alternative to the wilting test. Though this could be done by some vapour pressure method, the freezing-point method was preferred for reasons both of simplicity and of accuracy (Schofield, 1935).

As only a few soils had been examined it was decided to repeat the investigation on a larger scale. This was made possible through the kindness of Prof. Veihmeyer, who supplied the writer with a set of Californian soils in which the moisture equivalents and the "wilting coefficients" had been determined (Table II).

Table II

(Data supplied by Prof. Veihmeyer)

| | Soil type | *Moisture* equivalent | *Wilting* coefficient | Ratio |
|---|---|---|---|---|
| N | Oakley fine sand | 3·29 | 1·33 | 2·5 |
| HS | Hanford fine sandy loam | 8·84 | 3·99 | 2·2 |
| DS | Delano sandy loam | 9·09 | 4·14 | 2·2 |
| FS | Fresno sandy loam | 10·50 | 3·05 | 3·4 |
| M | Placentia loam | 10·52 | 3·70 | 2·8 |
| I | Columbia sand | 13·02 | 5·53 | 2·4 |
| TL | Tehama loam | 13·67 | 4·51 | 3·0 |
| 24 | San Joaquin sandy loam | 13·70 | 3·93 | 3·5 |
| 25 | Madera sandy loam | 14·56 | 3·62 | 4·0 |
| G | Columbia silt loam | 16·91 | 6·69 | 2·5 |
| CL | Columbia silt loam | 18·31 | 8·61 | 2·1 |
| H | Stockton clay adobe | 21·32 | 9·28 | 2·3 |
| S | Yolo silt loam | 21·35 | 10·13 | 2·3 |
| OL | Wooster silt loam | 23·36 | 6·12 | 3·8 |
| MG | Madera and Gridley loam | 25·63 | 10·25 | 2·5 |
| FAL | Farwell silt loam | 26·50 | 14·14 | 1·9 |
| $AK_2$ | Aiken clay loam | 28·93 | 20·75 | 1·4 |

These soils were particularly suitable for our purpose because (*a*) they include very different textural types, as shown by the moisture equivalents, (*b*) the ratio of the moisture equivalent to the "wilting coefficient" ranges from 1·4 to 4·0, (*c*) the "wilting coefficients" were determined using sunflower seedlings, whereas beans were the indicator plant used for the Portuguese soils first examined by Botelho da Costa (1933).

Fig. 1 gives the *pF* curves obtained for these soils by freezing-point (procedure *A*) between the moisture equivalent and the "wilting co-

efficient"; and Table III gives the $pF$ values at the "wilting coefficient" and at the moisture equivalent. To avoid confusion the intermediate

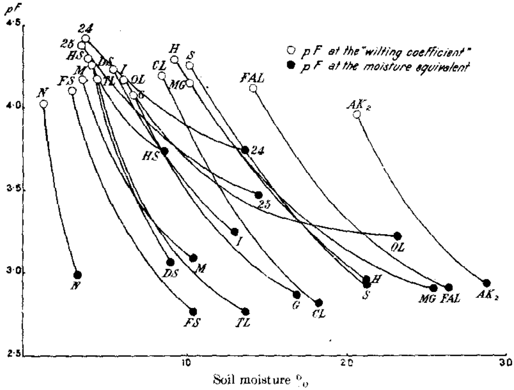


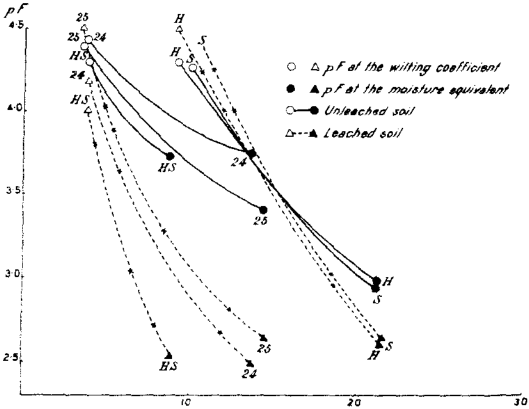

Fig. 1.

experimental points have been omitted from the diagram. The closeness of fit, however, can be seen in Fig. 2. and in the figure in the preceding paper, where the intermediate points have been inserted.

Table III

| Soil | $pF$ at the wilting coefficient | Salts % | Freezing-point depression of saturated soil | $pF$ at the moisture equivalent |
|---|---|---|---|---|
| 24 | 4·43 | 0·218 | 0·191 | 3·75 |
| 25 | 4·40 | 0·097 | 0·096 | 3·49 |
| HS | 4·30 | 0·088 | 0·076 | 3·73 |
| H | 4·30 | 0·040 | 0·026 | 2·96 |
| DS | 4·27 | 0·047 | 0·015 | 3·07 |
| CL | 4·25 | 0·025 | 0·013 | 2·80 |
| I | 4·25 | 0·051 | 0·021 | 3·25 |
| S | 4·25 | 0·039 | 0·018 | 2·93 |
| TL | 4·18 | 0·032 | 0·026 | 2·74 |
| M | 4·16 | 0·039 | 0·021 | 3·09 |
| OL | 4·16 | 0·092 | 0·061 | 3·22 |
| MG | 4·15 | 0·051 | 0·025 | 2·90 |
| FAL | 4·13 | 0·047 | 0·007 | 2·83 |
| FS | 4·11 | 0·023 | 0·021 | 2·76 |
| G | 4·08 | 0·056 | 0·013 | 2·83 |
| N | 4·01 | 0·018 | 0·016 | 3·00 |
| $AK_2$ | 3·97 | 0·047 | 0·019 | 2·91 |

The *pF* at the "wilting coefficient" varies between 4·0 and 4·4 (round figures), i.e. within the limits found in the preliminary investigation. As to the *pF* at the moisture equivalent, though for the majority of soils it approaches the theoretical figure 2·9, in some cases it is distinctly higher, as in soils 24, 25 and HS, resulting in rather "abnormal" *pF* curves. The most obvious explanation for this is a high salt content of the soils in qnestion. In order to investigate the influence of the salts, freezing-point measurements were carried out in some of the soils after leaching.



Soil moisture %

Fig. 2.

Leaching was carried out by twice shaking the soils with water, and filtering in large Buchner funnels. The soils used were the three giving the so-called abnormal *pF* curves (24, 25 and HS) and two heavier soils (H and S) which give normal *pF* curves. A comparison between the *pF* curves obtained before and after leaching is given in Fig. 2.

As expected, the *pF* curves of soils HS, 24 and 25 became "normal" after leaching. But whereas the *pF* at the "wilting eoefficient" dropped 0·3 in soil HS and 0·24 in soil 24, it rose 0·10 in soil 25.

In the heavier soils H and S, leaching caused a shifting of the *pF* curves, with the result of increasing the *pF* at the "wilting coefficient".

This change is apparently due to modifications of the natural structure by the leaching process. In other words, the *pF* of soils having an appreciable clay content was affected by the leaching process used. This result is in line with the effect of puddling a heavy clay soil upon its vapour pressure, found by Thomas (1921, 1924, 1928). Therefore the effect of salts upon the *pF* at the "wilting coefficient" cannot be investigated, as was hoped, by comparing the *pF* measured before and after leaching unless leaching is carried out by some process not affecting the structure. This effect of leaching is still noticeable in soil 25, whose *pF* at the "wilting coefficient" rose by leaching from 4·40 to 4·50. For some reason this effect was not so marked for soil 24, so that the effect of salts is clearly shown by the lowering of the *pF* at the "wilting coefficient".

The influence of the salts is still more clearly shown in the case of soil HS, in which the mechanical effect of leaching should not be very important in view of its coarser texture.

The salt content of all the soils was estimated by conductivity measurements (Table III). Admitting the total salt content is in the soluble state at the moisture equivalent, its influence on the *pF* measured by freezing-point at this moisture content can easily be calculated. Presuming 0·06° C. is the contribution of the insoluble soil material to the freezing-point depression, it was found that in all cases the salt content was high enough to account for the extra freezing-point depression observed, since the experimental values are in all cases lower than the calculated ones.

The salt content also affects the *pF* at the "wilting coefficient", though to a much smaller extent than the *pF* at the moisture equivalent, as should be expected. Thus soils 24 and 25 which have the higher *pF* at the "wilting coefficient" (respectively 4·43 and 4·4) also show the higher salt content (0·22 and 0·10%). A close relation between the salt content and the *pF* at the "wilting coefficient" is not to be expected because the "wilting coefficients" are widely different, so that the same amount of salt % of dry weight will correspond to very different concentrations of the soil solution at the "wilting coefficient" in the various soils. Thus soil OL, for instance, whose *pF* at the "wilting coefficient" is 4·16 has about the same salt content (0·09%) as soil HS, whose *pF* at the "wilting coefficient" is 4·30. But the concentration at the "wilting coefficient" is actually lower in soil OL, since its "wilting coefficient" is 50% higher.

As the "wilting coefficient" is roughly related to the heaviness of the soil, a better relation can be expected between the *pF* at the "wilting

coefficient" and the freezing-point depression (or the *pF*) at the moisture equivalent or the freezing-point depression measured in saturated soil. These are given in Table III. It will be seen that both the *pF* at the moisture equivalent and the freezing-point depression of saturated soil are higher in soil HS than in soil OL. The correlation is, however, still far from perfect, which is not to be wondered at, since the salts present may be different and the amount that goes into solution at different moisture contents is likely to vary from soil to soil. Nevertheless the figures given clearly show that part of the variation of the *pF* at the "wilting coefficient" can be accounted for by the presence of salts. For soils having a *pF* at the moisture equivalent under 3·4 or which, when saturated, give a freezing-point depression less than 0·07, the *pF* measured by freezing-point at the "wilting coefficient" never exceeds 4·3. More saline soils, however, (having a salt content over 500 p.p.m.), may show a higher *pF* at the "wilting coefficient". This must be taken into consideration in the indirect determination of the "wilting coefficient" by freezing-point.

Table IV

| Soil | "Wilting coefficient" observed (Veihmeyer) | "Wilting coefficient" determined by procedure A (factor 4·2) | Differences | "Wilting coefficient" determined by procedure B (factor 4·25) | Differences |
|---|---|---|---|---|---|
| N | 1·33 | 1·1 | −0·23 | 1·1 | −0·23 |
| FS | 3·05 | 2·8 | −0·25 | 3·0 | −0·05 |
| *25 | 3·62 | 4·9 | +1·28 | 4·9 | +1·28 |
| M | 3·70 | 3·6 | −0·10 | 3·6 | +0·10 |
| *24 | 3·93 | 5·9 | −1·97 | 5·9 | +1·97 |
| *HS | 3·99 | 4·4 | +0·41 | 4·4 | −0·41 |
| DS | 4·14 | 4·4 | +0·26 | 4·4 | +0·26 |
| TL | 4·51 | 4·5 | −0·01 | 4·4 | −0·11 |
| I | 5·53 | 5·7 | −0·17 | 5·8 | +0·27 |
| OL | 6·12 | 5·9 | −0·22 | 5·9 | −0·22 |
| G | 6·69 | 6·2 | −0·49 | 6·1 | −0·59 |
| CL | 8·61 | 8·8 | +0·19 | 8·7 | +0·09 |
| H | 9·28 | 10·1 | +0·82 | 10·1 | +0·82 |
| S | 10·13 | 10·4 | +0·27 | 10·3 | +0·17 |
| MG | 10·25 | 9·9 | −0·35 | 10·1 | −0·15 |
| FAL | 14·14 | 13·6 | −0·54 | 13·8 | −0·34 |
| $AK_2$ | 20·75 | 19·7 | −1·05 | 19·7 | −1·05 |
| Av. diff. (discarding saline soils) | | | ±0·35 | | ±0·32 |

* Soils having over 600 p.p.m. of soluble salts.

Table IV gives a comparison between the "wilting coefficients" and the moisture contents corresponding to *pF* 4·2 (the average *pF* at the "wilting coefficient" for all the soils examined). The agreement is good for all except the more saline soils. For these soils a somewhat higher factor would have to be used for determining the "wilting coefficient" by the

freezing-point method. Another alternative would be to do the freezing-point measurements after washing the soils by some means that would not alter their structure. More work is needed in this direction, and at the present state of our knowledge the freezing-point method can only be used confidently for the indirect determination of the "wilting coefficient" in soils having a salt content under 500 p.p.m., having $pF$ at the moisture equivalent under 3·4 and a freezing-point depression when saturated less than 0·07° C.

The results given above were obtained following procedure A[1] for the freezing-point measurements. For the purpose of indirect determination of the "wilting coefficient", procedure B[1] can be used with equally good results, by taking 4·25 as the average $pF$ at the "wilting coefficient" for non-saline soils, as shown in Table IV.

## Summary and conclusions

The results obtained in the preliminary investigation were entirely confirmed, the $pF$ at the "wilting coefficient", as measured by the modified freezing-point method, varied from 4·0 to 4·4 (round figures), with an average of 4·2.

The variation observed bears no relation to the soil texture, neither can it be explained by uncertainties in the freezing-point determinations which have proved to be accurately reproducible. Freezing-point measurements after leaching, conductivity measurements and freezing-point determinations in saturated soil and at the moisture equivalent proved that part of the variation is due to the presence of soluble salts, the more saline soils having a higher $pF$ at the "wilting coefficient". When the salt content does not exceed about 500 p.p.m. the influence of the salts is hardly detectable, and the $pF$ at the "wilting coefficient" lies between 4·0 and 4·3. Besides unavoidable errors in the wilting experiments[2] several other factors may account for this variation. They are all the factors that have any role in the "history" of the soil. In view of these uncontrollable sources of error a variation of 0·3 $pF$ units can be considered very small.

It can therefore be confidently concluded that in ordinary agricultural soils with a salt content of less than about 500 p.p.m. permanent wilting occurs when a critical $pF$ value (lying between 4·0 and 4·3) is reached.

[1] For descriptions of procedures A and B for determining the freezing-point depression see previous paper by Schofield & Botelho da Costa (1938).

[2] A difference of 1 % moisture content corresponds to a difference of 0·1–0·2 $pF$ units in the neighbourhood of the "wilting coefficient".

This knowledge affords a new indirect method of determining the "wilting coefficient" by freezing-point measurements in soils having less than about 500 p.p.m. of soluble salts. Procedures A or B are equally satisfactory for this purpose. As the *pF* curve is practically straight in the neighbourhood of the "wilting coefficient", two freezing-point measurements (round about 1–2° C. freezing-point depression) are enough for the indirect determination of the "wilting coefficient". The results are plotted using the *pF* scale, and the "wilting coefficient" is given by the moisture content corresponding to *pF* 4·2 if procedure A is used, or 4·25 when following procedure B. Extrapolations can be made if necessary from about *pF* 3·8.

The method is incomparably less laborious than the direct determination by wilting experiments.

Further investigation is needed in order to apply the method to saline soils.

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# AN APPARATUS AND TECHNIQUE FOR MEASURING THE RESPIRATORY EXCHANGE OF FED SHEEP OVER PERIODS OF FORTY-EIGHT HOURS

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(With Four Text-figures)

During the course of investigation into the energy metabolism of sheep it became evident that the apparatus which had been assembled in this laboratory (Lines & Peirce, 1931) for the study of the standard metabolism (fasting metabolic rate) was not suitable for the determination of the respiratory exchange over the much longer periods necessary for the investigation of the metabolism of fed animals.

However, the modified chamber, metering, sampling and analytical devices which are described below, render it possible to estimate the total energy expenditure of an experimental animal, subjected to the normal experimental routine and confined to the chamber for periods of 48 hr., with the degree of precision desirable for energy balance studies.

The respiratory exchange is measured by modifications of the open circuit method first used by Pettenkofer, the animal being placed in a chamber equipped to provide it with food, water and light, and with means to prevent the temperature and humidity reaching levels which would distress the occupant. The chamber is ventilated by a constant stream of air drawn through it, the total volume passed being determined by means of an apparatus which compensates for diurnal changes in temperature, pressure and humidity of the surrounding atmosphere. To accomplish this the mixed gases issuing from the chamber are brought to a constant temperature and saturated with water vapour before the volume is determined in standard wet meters. An aliquot sample is taken for analysis and the oxygen consumption and carbon dioxide excretion of the animal are calculated from the data so obtained.

## Details of construction and description of the apparatus

A diagram of the apparatus is shown in Fig. 1, to which the descriptions below refer. Constructional details are shown in Fig. 2.

(1) *The respiration chamber*

The respiration chamber consists of an airtight bell ($B_1$) of galvanized iron which, when lowered, shuts off its interior from the external atmosphere by dipping in a trough ($B_4$) which is filled with water and made continuous with a galvanized iron floor. This trough is partially filled with completely saturated sponge rubber in order to minimize diffusion through the water seal. The bell itself is covered on all sides with hair felt, 12 mm. thick, to diminish heat exchange with its surroundings; and a plate-glass window ($B_2$), 60 × 45 cm., is sealed into the roof to provide light, and to allow observations to be made. The inside of the chamber is

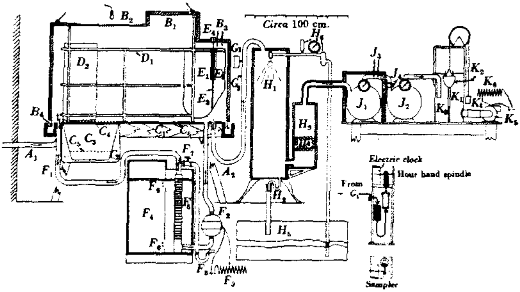


Fig. 1. Diagram showing flow of air in relation to components of apparatus. (Approximately to scale.)

lacquered white, and the felt on the outside is protected with a tight canvas jacket. A steel cable passing through pulleys is attached to a counterweight and to the four corners of the bell to facilitate raising it.

The metal framework ($D_1$) and enamelled shield ($D_2$) keep the animal's hindquarters over the wire grid ($C_4$), through which the excreta fall as voided into the pan ($C_3$). This pan is supported in a well ($C_2$) formed in the metal floor of the chamber. The metal framework is hinged at the front to permit removal of the pan. A zinc screen ($C_5$) within the pan effects a partial separation of urine and faeces, which facilitates cleansing. Slipping of the fore feet of the animal is prevented by the grooved wood flooring supported on the gastight metal base of the chamber.

The position of the feed box is adjustable so that animals of different lengths may be comfortably accommodated, and is arranged to hold

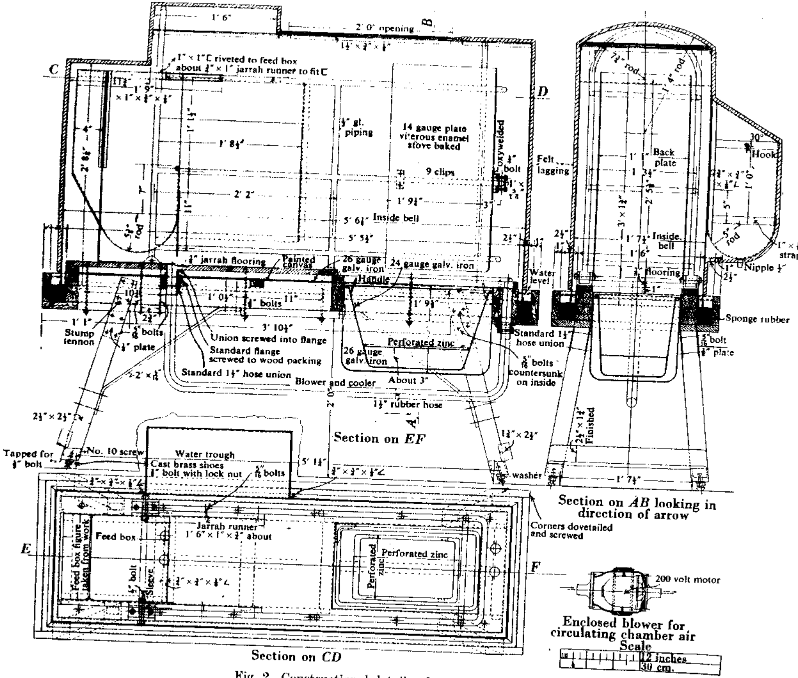


Fig. 2. Constructional details of respiration chamber.

rations for 2 days, the first being immediately accessible to the animal, the second, being held separately in the compartment ($E_2$), may be released at will by manipulating the wire ($E_4$) attached to the door ($E_3$).

All connexions to the respiration chamber are made through brass nipples sealed into the metal floor to minimize the risk of air leaks. Two such outlets are provided behind the enamelled iron shield ($D_2$) and two under the feed box. One of each pair is attached by means of a canvas and rubber hose (37 mm. bore) to the device for circulating and conditioning the air within the chamber. The outside air is drawn through a tube which passes through the outer wall of the laboratory and is attached to the other rear nipple. The mixed chamber gases are removed through the remaining nipple in front. The necessity for keeping the volume of the chamber as small as is compatible with the comfort of the animal was discussed elsewhere (Lines & Peirce, 1931), and this was continually kept in mind when designing it.

### (2) *Air conditioning*

The circulation of the air within the chamber is maintained by a blower ($F_2$) with its motor enclosed within the volute. Air is drawn from the rear of the chamber at the rate of about 1000 l. per min., and, after passing through the conditioning apparatus, returns to the front end of the chamber. The conditioning apparatus consists of a motor car radiator ($F_5$) immersed in an insulated tank filled with water, which is cooled by placing blocks of ice in the compartment ($F_4$), circulation of the cold water being maintained by convection through the openings ($F_6$) between the two compartments of the tank. Condensed moisture which accumulates during the conditioning is allowed to drain off through the trap, $F_8$. The extent to which the air is cooled and dried may be controlled by manipulating the by-pass tap ($F_7$) and rheostat ($F_3$), which is in series with the blower motor.

Wet and dry bulb thermometers are inserted through the nipples $B_3$, and from these the conditions within the chamber may be determined and corrected if necessary.

### (3) *Precautions against leaks in the apparatus*

As the barometric pressure within the chamber is maintained about 0·1 mm. mercury below that of the surrounding atmosphere as a result of the suction set up by the ventilation fan, errors arising from leaks are minimized. The only portion of the whole circuit in which there is any positive pressure is in the tube by which the air from the blower returns

to the chamber after passing through the conditioning apparatus, and here leaks are rigorously excluded.

### (4) *The sampling device*

The mixture of air and respired gases is withdrawn from the chamber through the pipe $A_2$, which has two sampling points, ($G_1$) and ($G_2$). An aliquot sample of about 70 l. is withdrawn through $G_2$, and analysed for carbon dioxide and methane by means of the apparatus described by Lugg (p. 688 of this issue).

The sampling device ($G_1$) consists of two glass cylinders of 5 cm. internal diameter and 12 cm. long, each with its axis vertical. One, which serves to collect the sample, is fixed, while the other, which is lowered at a constant rate by means of an electric clock, serves to withdraw the mercury from the sampling cylinder. These cylinders are constructed with plate-glass ends cemented in position as indicated in the diagram. They are connected by a length of heavy-walled rubber tubing. The load on the delicate mechanism of the electric clock is kept small and constant by suspending the receiving cylinder on a spiral spring of such a strength that the weight of mercury in 1 cm. depth of the cylinder extends the spring 2 cm. The gases entering the sampler are dried by passing them through concentrated sulphuric acid. By this means the sample is aspirated at a constant rate, and as the flow of gas through the pipe $A_2$ is kept constant, the result is a true aliquot of the mixed chamber gases drawn through the apparatus.

### (5) *Apparatus for saturating and adjusting temperature and metering the chamber gases*

The pipe ($A_2$) leads into the top of a spray tower ($H_1$), 120 cm. high and 30 cm. in diameter, where the gases are saturated and brought to the temperature of the spray water prior to metering. Two spray jets at the top of the tower are supplied with water from a well-insulated underground tank at the rate of 1500 c.c. per min. and at a pressure of 2 atm. by a small boiler feed pump. The water returns to the tank through the trap $H_2$. At a point 10 cm. above the bottom of the tower the gases are led off into a cylinder, 60 cm. high and 20 cm. in diameter, where any spray entrained is caught in a layer, about 10 cm. deep, of glass spheres (12 mm. in diameter), which rest on a perforated shelf. This part of the apparatus is insulated by means of a cover of hair felt, 12 mm. thick.

### (6) *The meters*

The gases from the spray tower are then led through an insulated pipe to the standardized wet gas meters, which, in our experience, have been found to maintain excellent accuracy for considerable periods providing that the water level is kept constant. This is achieved by means of a syphon

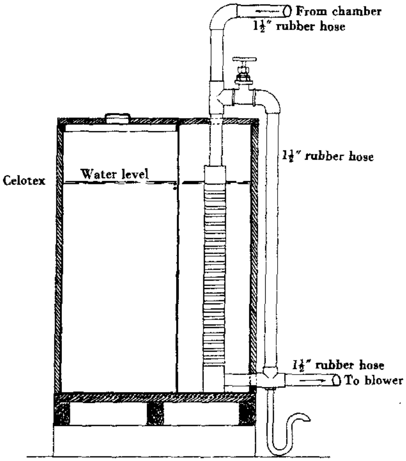


Fig. 3. Diagram of apparatus used to cool and dry air recirculated from chamber. (The tank is approximately 50 cm. wide.)

overflow discharging outside the insulated box in which the first meter is set np. Wet and dry bulb thermometers ($J_3$) and a valve which serves to regulate the flow of gas through the whole system are fixed in the tube connecting the first and second meters. The meters are calibrated at frequent intervals.

### (7) *Apparatus for maintaining constant suction*

A constant negative pressure of 2 mm. of mercury is maintained within the vessel ($K_1$) by means of the centrifugal blower ($K_5$) and the weight-loaded relief valve $K_2$. The speed of the blower is regulated by the

rheostat, $K_6$, so that about three times as much air is drawn from the vessel $K_1$ as enters it from the meter $J_2$. The remainder of the air finds its way through the valve $K_2$. The bobbin of this valve, the excursions of which compensate for change in density of the room air and alterations in the speed of the blower, is held central to the hole in the top of the vessel

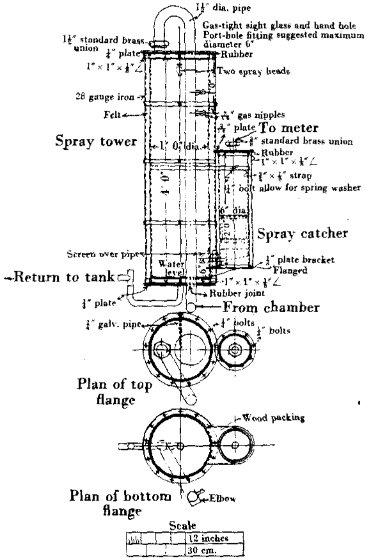


Fig. 4. Constructional details of tower for saturating and equalizing the temperature of the gases before metering.

$K_1$ by means of the guide $K_3$. Experiment showed that the flow lines set up by the shape of the bobbin are important. A shape which gives satisfactory results had its upper surface formed by rotating the hyperbola $(x-0{\cdot}5)\,y=2{\cdot}5$ cm.$^2$ from $x=1$ to $x=5$ around the $y$ axis, and its lower surface by rotating the parabola $(x+1)^2=4y+36$ cm.$^2$ between $x=0$ and $x=5$ around the same axis. A bobbin of this size is used with an opening 8·5 cm. in diameter.

## Examination of the validity of the aliquot sample

As a constant suction is maintained on the valve $J_4$, and as the mixed chamber gases which enter this are maintained at a constant humidity and temperature, the rate or flow is uniform, and so samples drawn off at a constant rate are true aliquots. Should the rate of ventilation and the rate of respiratory exchange of the subject alter simultaneously an error is introduced in the sampling. Normally this is very small. If the rate of ventilation be $v$ and of respiratory exchange $c$ litres per minute respectively for $t$ minutes, and these rates then change to $v'$ and $c'$ for $t'$ minutes, it will be seen that the relative error between the apparent and real respiratory exchange would be

$$\frac{tt'}{t+t'} \times \frac{(v-v')\,(v'c-vc')}{vv'\,(ct+c't')},$$

an expression which reaches a maximum when $t=t'$ and zero if $v=v'$, and even should conditions involving changes of a magnitude of 10% occur in $v$ and $c$ in opposite directions when $t=t'$ an error of less than 1% would result. Such a magnitude of change rarely, if ever, occurs during operation of the apparatus.

## The technique used in measuring the respiratory exchange

Training the sheep so that they experience no discomfort or alarm during their sojourn in the chamber is a primary and essential consideration, and to ensure this the experimental animals were submitted to the treatment described by Marston (1935). Mature ewes trained in this way exhibit no sign of excitement when in the chamber and continue to consume the whole of the ration offered, upon which they had been fed for some weeks previous to the determinations. The experimental animals spend the whole time during which they are confined in the chamber either standing or reclining as peacefully as if they were in their normal environment. Under such conditions the respiratory exchange from day to day has been observed to remain extraordinarily constant.

After ascertaining that the mechanical contrivances are functioning perfectly, the rations for two days are placed separately in their respective compartments of the feed box. The excreta pan is washed out with a solution of 10% thymol in toluene. About its usual feeding time the animal is placed in the cage with the feed box in position. The bell is then lowered and the circulating blower started.

As the free volume of air within the chamber is nearly equal to the

amount which would pass through in 25 min. at the usual rate of ventilation, equilibrium is established by circulating the mixed gases for this period before commencing ventilation. Sampling is begun 10–15 min. later to allow the animal to adjust for the slightly increased carbon dioxide tension of its environment before the collection is started. During this interval the sampling device and tubes are well washed out with the gases coming from the chamber, the rate of ventilation adjusted and the various thermometers read. The meters are then read, the time noted, and the collection of the sample started.

The behaviour of the experimental animal and the working of the apparatus is noted periodically throughout the ensuing day. Experience has shown, however, that adjustments are rarely necessary and that trained animals exhibit no sign of discomfort or uneasiness while undergoing the test. In dull weather a 50 W. electric lamp is lowered directly over the glass window at the top of the chamber and kept alight for 8 hr. during the day time. The wet bulb temperature within the chamber is kept below 20° C. by adjusting the flow through the air-conditioning plant.

After 24 hr. the total volume of gas which has passed is read on the meters and the aliquot sample drawn off into a Bailey (1921) gas bottle and sampling resumed at once. The second day's ration is then liberated by manipulating the sliding door of the food bin. After the lapse of a further 24 hr. the necessary readings are taken and the experiment stopped. Records are kept of the wet and dry bulb temperature of the chamber gases as they leave the insulated meter, and the total volume of gas which has passed through the chamber is redneed to normal temperature and pressure and corrected for the volume of aqueous vapour it contains by means of the formula

$$V = vM \times \frac{273}{273+t} \times \frac{d}{760},$$

where $V$ is calculated volume of air at 760 mm. Hg and 0° C.,

$v$ is volume recorded by meter,

$M$ the meter calibration factor,

$t$ the mean dry bulb temperature,

$d$ the pressure of dry air, i.e. the difference between the actual barometer and the pressure of aqueous vapour in the air coming from the meter as ascertained from Assman's tables.

These data are collected on a prepared form, an example of which is shown in Table I.

Table I. *Form of record and example of observations used to reduce metered volume to standard conditions*

Sheep $W_3A_{43}$. Sex ♀. Live wt. (kg.) 33·1. Date 8. vii. 35

| | | | |
|---|---|---|---|
| Entered chamber | 12·17 | Meter 1 | Meter 2 |
| Blower started | 12·42 | 8445·5 cu. ft. | 3794·5 cu. ft. |
| Sampler started | 12·52 | 8454 | 3803 |
| Sampler stopped | 36·52 | 9786 | 5152 |
| Volume sampled | | 1332 cu. ft. | 1349 cu. ft. |

Volume sampled in cu. ft. $\times 28·32 = v$ in litres. $M = 1·01$.

| | Meter ° C. | | Chamber ° C. | | |
|---|---|---|---|---|---|
| Time | Wet | Dry | Wet | Dry | Remarks |
| 12·20 | — | — | 13·1 | 13·0 | Eating. Light on |
| 12·44 | 10·1 | 10·3 | 13·4 | 13·8 | |
| 15·00 | 10·4 | 10·5 | 14·3 | 15·2 | Eating |
| 17·15 | — | — | — | — | Light off |
| 33·15 | 10·9 | 10·9 | 12·7 | 13·0 | Standing |
| 36·50 | 11·8 | 11·9 | 14·5 | 15·0 | |
| Mean | 11·0* | 11·1† | 13·5 | 14·0 | |

Barometer mean 761·8 mm. Hg.

Aq. vap. 11° C. 9·7 mm. Hg. Dry air pressure 752·1 mm. Hg $= d$.

$37{,}720 \times M \times \frac{273}{284} \times \frac{752}{760} = 36{,}250$ l. dry 0° C. and 760 mm.

Add $CH_4$ sample. 66 l. = 36,315 l. (= $V$).

* Mean dry minus mean difference between wet and dry.
† Computed mean for 24 hr.

ANALYSIS OF THE ALIQUOT SAMPLE

The method of sampling outlined above results in the collection over mercury of a dry aliquot sample of the gases coming from the chamber. After each 24 hr. sufficient of this for four analyses is transferred to a Bailey bottle over dry mercury, where it is held until analysed. This method of storing results in little, if any, change in composition. The sample is transferred to the apparatus for analysis through a three-way glass tap and subsequently analysed in a modified Haldane (1920) apparatus[1] fitted with a Carpenter (1923) burette and a Strieck (1928) oxygen pipette. The apparatus was further modified by fixing to the bottom of the measuring burette a trap into which any foreign materials finding their way into the mercury could be washed. A few drops of a 1% solution of citric acid are placed on the mercury so as to moisten the burette during the analysis. This keeps the gases saturated and avoids errors in the $CO_2$ estimation which sometimes arise through the solution of alkali from the glass. Experience stresses that special care must be

[1] Constructed for this laboratory by Messrs Bleckmann and Bürger, of Berlin.

devoted to greasing all stopcocks, and for this purpose a pure vaseline solution of raw rubber is useful. Notwithstanding meticulous care, a film of this material occasionally finds its way into the burette. When this happens it must be cleaned out with alcohol and toluene and all traces of the solvents removed by several washings with water before proceeding with the estimations.

While the procedure adopted for analysis is essentially that described by Carpenter *et al.* (1929) certain changes in handling the apparatus have been made.

If the apparatus is to be left unused for more than a few hours the compensating burette is opened to the air and the taps leading to the measuring burette and absorbing pipettes are closed. Before an analysis is begun the temperature in the water jacket is adjusted to that of the room by means of a small immersion heater and the compensator is closed. The nitrogen remaining in the apparatus from the last determination is used to wash out the dead space by passing it twice into the potash, five times into the pyrogallate and twice again into the potash; this removes any residual oxygen in the apparatus. The burette itself is then washed out with room air to equilibrate the dilute citric acid solution therein with air instead of the nitrogen which has remained above it from the previous determination. The connexions to the sampling bottle are washed out with part of the gas to be analysed, using the mercury levelling bulb to evacuate them each time. The burette is then filled with the sample under slight positive pressure, the water-jacket agitated for a minute or so and the excess pressure allowed to escape. The burette is then connected to the manometer, which is adjusted after agitating the water in the jacket for a further three minutes. The volume of gas in the burette is then read and recorded (as $i$ in Table II). After passing the gas six times into the caustic potash pipette the burette is again read, using the same precautions as previously stated, and the reading recorded (as $CO_2$ in Table II).

When absorbing the oxygen it was found that the number of passages into the pyrogallate solution could be reduced to sixteen without sacrifice of accuracy, providing that the caustic potash solution was raised to within 5 mm. of the tap of the carbon dioxide absorption pipette as soon as the main bulk of the oxygen was absorbed. The procedure for the absorption of oxygen now adopted in this laboratory is to raise the level of the potash after four passages of the gas into the pyrogallate. The gas is then passed six times further into the pyrogallate, twice into the potash, and this is followed by six more passages into the pyrogallate, and finally,

twice again into the potash. The levels are then adjusted and the meniscus of the mercury read and recorded as $O_2$, and the level of the water read and recorded as $H_2O$ in Table II. A second analysis is then carried out and if the two results differ, a third determination is made and the mean of the two most concordant results taken. The results rarely vary within 0·05 % of the amount of oxygen and within 1 % of the amount of carbon dioxide determined. If the three determinations are discordant the cause is determined and rectified, and the analyses repeated.

Table II. *Reduction of burette readings of gas analysis to percentages and calculation of respiratory exchange. Analysis of aliquot sample*

Animal No. $W_8A_{48}$. Date 8. vii. 35.

| Assay I | Burette | Corrected | Ratio | Difference | % |
|---|---|---|---|---|---|
| *i* | 0·182 | 0·181 | — | — | — |
| $CO_2$ | 0·980 | 0·974 | 0:793 | 0·002 | 0·795 |
| $O_2$ | 21·086 | 21·073 | 20:099 | 0·066 | 20·165 |
| $H_2O$ | 21·130 | 21·217 | 78:783 | 0·257 | 79·040 |
| + $H_2O$ | 0·100 | Total | 99:675 | 0·325 | 100·000 |
| **Assay II** | | | | | |
| *i* | 0·280 | 0·278 | — | — | — |
| $CO_2$ | 1·080 | 1·073 | 0:795 | 0·003 | 0·798 |
| $O_2$ | 21·170 | 21·156 | 20:083 | 0·086 | 20·169 |
| $H_2O$ | 21·220 | 21·306 | 78:694 | 0·339 | 79·033 |
| + $H_2O$ | 0·100 | Total | 99:572 | 0·428 | 100·000 |

| Mean | % | Analysis | % |
|---|---|---|---|
| $CO_2$ | 0·796 | $CO_2$ | 0·796 |
| $O_2$ | 21·167 | $O_2$ | 20·167 |
| $N_2 + CH_4$ | 79·037 | $N_2$ | 78·980 |
| | | $CH_4$ | 0·056 |

Urinary N 8·23 g.

| | % |
|---|---|
| $O_2$ equivalent to N | 20·932 |
| $O_2$ found | 20·167 |
| % O deficit | 0·765 |
| $CO_2$ found | 0·796 |
| $CO_2$ in atmosphere | 0·030 |
| % $CO_2$ produced | 0·766 |

Total volume (from Table I) 36,315 l. dry 0° C. 760 mm.

Caseous exchange

| | |
|---|---|
| $CO_2$ produced | 278·2 l. |
| $O_2$ deficit | 278·0 l. |
| $CH_4$ produced | 20·3 l. |

The burette readings are corrected from the calibration table, the volume of the water which wets the inside of the burette bulb (0·10 %) allowed for, and added to the corrected reading of the water meniscus. The successive differences between the corrected values of "*i*, $CO_2$" and

"$O_2$" give the relative volumes of carbon dioxide and oxygen in the sample, whilst 100 minus the corrected reading of the water meniscus gives the volume of nitrogen plus methane. The relative values so obtained are added together and the difference between their sum and 100 are distributed proportionally between them. The percentage of methane, as determined in a separate aliquot, is deducted from the nitrogen plus methane figure to obtain the nitrogen (i.e. $N_2$ plus rare gases). Any hydrogen evolved as a result of bacterial activity in the rumen of the experimental animal would appear in such a determination as nitrogen. However, careful search by our colleague failed to find any measurable amount of hydrogen in the ventilation gases arising from the animals under experiment.

The mean assay of dry outside air at Adelaide was found to be $CO_2 = 0{\cdot}030\%$; $O_2 = 20{\cdot}945\%$; $N_2$ etc. $= 79{\cdot}025\%$, which compares with $0{\cdot}031\%$ $CO_2$ and $20{\cdot}940\%$ $O_2$ as found in U.S.A. by Carpenter (1937). The amount of oxygen entering the chamber is calculated from the metered volume of gases leaving it, by multiplying this figure first by the percentage of nitrogen found in the aliquot sample and then by the $O_2 : N_2$ ratio of the outside air, namely, 20·945:79·025. The amount of oxygen leaving the chamber is obtained by multiplying the volume of gases by the oxygen in the aliquot sample. The difference between these gives the amount consumed by the animal. The carbon dioxide produced is calculated from the percentage found in the aliquot sample minus the $0{\cdot}030\%$ of the ingoing air. This procedure for calculating the gaseous exchange is essentially that suggested by Benedict *et al.* (1934).

## CALCULATION OF THE TOTAL HEAT PRODUCTION IN THE FED SHEEP FROM THE MEASUREMENTS OF GASEOUS EXCHANGE

The total gaseous exchange as measured in this apparatus includes the large volume of carbon dioxide and methane which is formed as a result of bacterial digestion within the alimentary tract of the animal. There seems to be no general agreement between various workers as to the ratio of the carbon dioxide to the methane produced during bacterial digestion of carbohydrates, etc., in the tract. The fermentation is, however, exothermic and the temperature of the rumen, in which organ the main cleavage occurs, was found by Krzywanek (1929) to be about 0·5° C. higher than that of the rectum. The method of A. C. Andersen (1920) is used to calculate the total heat production, which includes that arising from fermentation, consumption of oxygen and production of

carbon dioxide and methane. This method is preferred to that of Benedict *et al.* (1934) as it appears to be thermodynamically valid. Andersen adds the amount of oxygen necessary to burn the methane production to the amount of oxygen consumed by the animal, and the resulting carbon dioxide is added to that evolved by the animal. From these augmented totals, the equivalent calories are calculated by the method first used by Zuntz & Schumburg amended in accordance with more recent measurements of the heat of combustion of polysaccharides and fatty acids. The difference between the heat produced when all combustible substances are reduced to $CO_2$ (gas) and $H_2O$ (liquid), and the heat of combustion of the methane under the same conditions, gives the heat produced by the animal and its symbiotic flora during the period in the chamber. The non-protein respiratory quotient is estimated from the urinary nitrogen excretion over the same period. The method is summarized in the following expression:

If $C'$ = litres of $CO_2$ produced,
$M'$ = litres of $CH_4$ produced,
$O'$ = litres of $O_2$ consumed,
$U$ = g. of urinary N over the same period,

then the non-protein respiratory quotient is

$$\frac{C'-6U+M'}{O'-7\cdot5U+2M'}=R,$$

and the energy output is

$$(O'-7\cdot5U+2M)\,(4\cdot660+1\cdot29\,\overline{R-0\cdot707})-9\cdot44M+35U.$$

The factors used for calculating the heat produced by oxidation of protein are computed for a plant proteiu which contained 14 % of nitrogen instead of the conventional 16 %.

An example of the method of calculation is set out in Table III, in which form the data collected in this laboratory is filed.

Table III. *Computation of heat output in kilogram calories from the gaseous exchange in litres per day and the urinary excretion of nitrogen in grams per day*

| | | | |
|---|---|---|---|
| $CO_2$ | 278·2 l. | $O_2$ | 278 l. |
| $CH_4$ | 20·3 l. | N | 8·23 g. |

$$\text{Non-protein R.Q.}=\frac{278\cdot2-6\times8\cdot23+20\cdot3}{278-7\cdot5\times8\cdot23+40\cdot6}=\frac{249\cdot1}{256\cdot9}=0\cdot97.$$

$$\text{Kg. cal.}=256\cdot9\times5\cdot00-9\cdot44\times20\cdot3+35\times8\cdot23=1381.$$

### TESTING THE ACCURACY OF THE APPARATUS

Exhaustive tests have been made from time to time to gauge the accuracy of the results obtained with the apparatus. These were carried out in collaboration with my colleagues. The amount of oxygen consumed and carbon dioxide evolved when alcohol or acetone was burned in a lamp placed within the chamber was determined and compared with the theoretical quantities calculated from the amount of fuel burnt. A small glass tube fitted with an asbestos fibre wick served as the lamp, whilst the fuel was kept at a constant level by means of a calibrated Marriott bottle. The wick was adjusted so as to ensure that the oxygen consumed and carbon dioxide evolved were roughly equivalent to those amounts produced by the sheep. The procedure followed was identical with that adhered to when estimating respiratory exchange of the sheep.

The following table outlines the actual findings of typical tests:

| Duration hr. | Burnt | $CO_2$ found | $CO_2$ theory | % | $O_2$ used | $O_2$ theory | % |
|---|---|---|---|---|---|---|---|
| 24 | 154·8 g. acetone | 180·7 | 182 | 99·3 | 242 | 243 | 99·5 |
| 21 | 164·6 g. ethanol | 159·8 | 160·4 | 99·6 | —* | — | — |
| 20 | 126·6 g. ethanol | 123·1 | 123·4 | 99·8 | 182·6 | 182·3 | 100·2 |

* Not determined.

### SUMMARY

The gaseous exchange of the sheep can be measured in the apparatus described with considerable accuracy and good agreement obtained with the energy absorbed from a maintenance diet. The apparatus automatically compensates for diurnal changes in temperature and humidity, and maintains a constant rate of ventilation.

The precautions necessary to ensure that the metabolism as measured is a good sample of that of the animal when subsisting on the diet, consist of control of temperature and humidity within the chamber and training the animal so that it is free of psychic strain during the period of measurement. It was found that the sheep takes 14–20 days to adjust its energy turnover to a change in diet.

Modification in structure and operation of the Haldane-Carpenter gas analysis apparatus are described in detail.

The method of A. C. Andersen is used to comput , the energy metabolism and respiratory quotient from the gaseous exchange and methane production.

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# A NOTE ON THE ESTIMATION OF THE SULPHUR CONTENT OF FODDER AND EXCRETA

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EXIGENCIES of space do not allow a full discussion of the extensive literature which deals with the difficulties encountered when attempts are made to estimate the sulphur content of organic materials. Many methods have been proposed and most have been the subject of criticism from time to time.

The main difficulties to be overcome are those associated with the complete oxidation and quantitative retention of sulphur during the destruction of the organic matter. General experience suggests that wet digestion methods, utilizing strong oxidizing reagents such as nitric and perchloric acids, either in open vessels or in closed Carius tubes, often result in losses of sulphur, presumably through the formation of substituted sulphonic acids formed during the early stages of oxidation. These are volatilized from the open digestion flasks. Errors thus introduced may be overcome if Kahane & Kahane's (1934) suggestion is followed and the evolved gases passed through a solution of iodic acid, but the procedure is time-consuming and unhandy when dealing with samples containing small amounts of sulphur.

Oxidation involving fusion with sodium peroxide is capable of yielding reasonably accurate results with some classes of material. Such methods are, however, uncertain even in the most careful hands. Oxidation with copper and ammonium nitrate in the presence of excess sodium chloride, as was suggested by S. R. Benedict (1909) and later modified by Denis (1910) for the estimation of total sulphur in the urine of mixed feeders, is more easily controlled. This method has been adapted for use with other materials (Frear, 1930). When applied to the urine of sheep, however, it has proved by the following investigations to be capable of yielding misleading results through the loss of sulphur.

All dry fusion methods share an undesirable complication. The relatively enormous excess of salts present in the final solution renders

doubtful the composition of the barium sulphate precipitate owing to the possibility of occlusion and of double salt formation.

The classical Fresenius method of burning the sample in a stream of air in a combustion furnace and collecting the sulphur-containing gases in strong oxidizing reagents has much to recommend it on *a priori* grounds. A number of critical studies, extending from Barlow (1904) to Sielisch & Sandke (1932), have amply demonstrated its reliability, but the manipulations involved and the time consumed in the process detracts from its general usefulness.

The manipulations may be simplified by burning the material in compressed oxygen, following Bethelot's original suggestion that his bomb calorimeter might be used for the estimation of sulphur in organic materials. The experiences of Le Clerc & Du Bois (1904), who were embarrassed by the formation of lead sulphate through the interaction of nitric-sulphuric mixtures with the lead gasket of their bomb, caused this method to fall into disuse for many years.

Following an investigation into its potentialities, which led to certain simple modifications, this method has been used in the Nutrition Laboratory for several years and has been found to be the only method of a number examined which would yield accurate results with a diversity of materials. We thus agree with Garelli & Saladini (1931) and advocate its use.

The critical assays of fodder, faeces and urine mentioned below are typical of the information which led us to form the above opinion.

## Experimental

The materials used in these analyses were collected and treated as previously outlined (Marston, 1935*a*). The fodder, which consisted of a mixture of chaffed lucerne hay and wheaten straw, was sampled and ground in a Wiley mill until it all passed a 0·5 mm. mesh, moisture checks being carried out before and after grinding. The sheep faeces were treated similarly. The finely ground powders were spread on glazed paper and allowed to come into equilibrium with the laboratory air. Samples for all analyses were then drawn into tared weighing bottles, accurately weighed, and the water content determined by drying to constant weight *in vacuo* at 100° C. A measured volume of urine was evaporated in a serum drier at 40° C. to the consistency of thick treacle, and samples for the determinations involving the bomb and sodium peroxide methods were drawn from this. Measured volumes of urine were used for the

Benedict-Denis and wet digestion methods. The determinations were conducted as follows:

(1) *Benedict-Denis*

Approximately 2 g. of the dry material (or 50 ml. of urine) were transferred to a new silica basin of 200 ml. capacity and well mixed with 50 ml. of Benedict-Denis solution made up from highly purified reagents. The mixture was then evaporated to dryness without boiling on an electric hot plate. The resulting mass was wetted with about 100 ml. of water and again taken to dryness so as to ensure that the individual particles of the solid materials were impregnated with the reagent. The heat was then increased until combustion commenced. This proceeded quietly in all cases. The dish was then transferred to an electric muffle and heated to 400° C. for a few minutes. When cool, the black residue was digested with excess of 6 $N$ HCl, washed into a 1 l. pyrex beaker and cautiously taken to dryness at 100° C. It was then taken up in 250 ml. of water, filtered and washed, and the volume made up to about 400 ml. The acidity was adjusted to approximately 0·02 $N$ with hydrochloric acid and the precipitation conducted as below.

(2) *Sodium peroxide fusion*

Approximately 2 g. of the material, accurately weighed, was transferred to a nickel crucible of 100 ml. capacity, 5 g. of G.R. sodium carbonate added and the contents well mixed. The mixture was then damped with 2 ml. of water and 5 g. of G.R. sodium peroxide cautiously mixed in until a granular mass resulted. When dealing with the evaporated urine no further water was added at this stage. The lid was fixed and the crucible placed in a cold electric muffle and the heat raised slowly to 500° C. At this point the crucible was removed and when cool the top of the melt was covered with a further 2 g. of sodium peroxide and the crucible replaced in the hot muffle for a further 10 min. After cooling, the crucible was transferred to a 1 l. pyrex beaker, covered with a clock glass, and the contents dissolved in water. Excess of hydrochloric acid was then introduced and the solution washed through a filter paper. The acidity was adjusted to 0·02 $N$ and precipitation conducted as below.

(3) *Wet digestion with perchloric-nitric acid mixture*

Approximately 2 g. of the material, or 50 ml. of urine, was transferred to a 100 ml. pyrex Kjeldahl flask. Fifty ml. of pure nitric acid, S.G. 1·30, was added and the mixture heated gently for 12 hr. A further 50 ml. of nitric acid, together with 20 ml. of 70 % perchloric acid, were introduced

and the digestion continued for another 12 hr. The colourless solution which, in the case of the faeces and fodder, contained an abundant suspension of silica was transferred to a dish and cautiously evaporated to dryness on an electric hot plate. The residue was taken up in dilute hydrochloric acid and washed through a filter paper, the acidity adjusted to 0·02 $N$ and precipitation carried out as below.

### (4) *Combustion in compressed oxygen in the Emerson Bomb*

Approximately 2 g. of the dry material (fodder or faeces) were compressed into a hard tablet around a fuse wire by means of a die described elsewhere (Marston, 1935*b*), or about 2 g. of evaporated urine were transferred to a bomb capsule. The material was then weighed on the capsule, upon which it was later ignited in the bomb, allowance being made for the iron fuse wire, the weight of which was previously estimated. The capsule and its contents were then set up in the bomb and the fuse wires were fixed in position. *The lead washer of the bomb was then completely covered with a pure tin gasket,* and the bomb assembled and tightly screwed down. Pure oxygen was then let in to a pressure of twenty atmospheres, the valves closed and the bomb immersed in water to detect any leak of oxygen. The bomb was then fired electrically. If this procedure is followed complete combustion results. The silica and basic elements become fused into clear glass beads and the iron wire completely transformed to $Fe_2O_3$. A large proportion of the nitrogen in the sample is burned to nitric acid and all of the sulphur is transformed into sulphate. Careful search in preliminary experiments failed to detect any volatile sulphur compounds in the internal atmosphere of the bomb after firing. In consequence, the precaution of washing the residual gases, which was first resorted to, was abandoned and the procedure of merely opening the valve to relieve the pressure was adopted. The contents of the bomb are then completely washed out into a pyrex beaker, acidified with excess hydrochloric acid and taken twice to dryness at 100° C. to free from nitric acid. The residue was taken up with hydrochloric acid, washed through a filter paper and the acidity adjusted to 0·02 $N$ in a final volume of about 250 ml. The precipitation was conducted as follows:

### (5) *Precipitation and estimation of* $BaSO_4$

The perfectly clear and faintly acid solutions were brought to incipient boiling on an electric hot plate. Excess of hot barium chloride solution was added and the beakers were covered with watch glasses. They were allowed to remain at approximately 100° C. for a further 2 hr., when

the current to the heaters was switched off and the temperature allowed to fall slowly over-night. Next day the major quantity of the supernatant solution was sucked off through a bent capillary and the precipitate quantitatively transferred on to a Whatman 44 paper, where it was thoroughly washed. The paper and precipitate were then dried in a platinum crucible and ignited in an electric muffle. The residue was then very carefully "fumed off" with a drop of pure nitric acid and re-ignited at a temperature of about 500° C. The lid was then affixed and the crucible and its content allowed to cool in the air of the balance room. This latter procedure is important, as considerable errors are encountered if weighing is carried out before the crucible is allowed to come into equilibrium with the air surrounding it. Cooling in a desiccator and weighiug in a "dry" atmosphere of a balance case in which desiccating agents are exposed is not to be recommended when small differences are sought. The humidity of presumably dry air in the balance case is quite sufficient at times to lead to considerable errors. The difference in weight of the ignited crucible and its contents in equilibrium with the air of the balance room and the weight of the cleaned and ignited crucible similarly treated, proved by far the most reliable method for estimating the relatively small amounts of barium sulphate. Meticulous care was exercised in all the manipulations discussed above and the highest quality guaranteed reagents were used throughout, checks being made of the small amounts of extraneous sulphur introduced in the reagents.

## Discussion

The analytical data tabulated below are sufficient to make clear the order of error which may be unwittingly encountered when certain methods, which have found wide application, are used for the estimation of sulphur in organic materials.

While the Benedict-Denis method is claimed to be capable of estimating the total sulphur content of the urine of mixed feeders it is evidently not applicable to the urine of sheep. In the above analyses the recovery was only about two-thirds of the sulphur demonstrated to be present by the bomb technique. The more general application as advocated by Frear (1930) is by no means sure. Painter & Franke (1936) applied it to cereals, cereal by-products, wheat proteins and casein and found that the recoveries of sulphur were poor. These investigators showed that while reasonably good, though not quantitative recoveries, resulted when the method was used to estimate the sulphur present in cystine, only about one-third of the sulphur of methionine was preci-

pitated by barium sulphate, after treatment with the Benedict-Denis oxidizing reagent. They suggested that this was the reason why the method failed when applied to materials coutaining protein. While this may be generally correct, the reagent when used as outlined above gave good results in the author's hands when used for the estimation of the sulphur content of the particular sample of protein-containing fodder examined. It failed, however, when applied under identical conditions to sheep's faeces and urine, and doubt is thus cast on its general usefulness.

The sodium peroxide fusion failed badly when applied to sheep's uriue which had been evaporated at low temperature to the consistency of treacle. The recoveries of sulphur from the fodder and sheep's faeces were by no means quantitative. It is difficult to comprehend the incompleteness of oxidation under the conditions outlined in the Official Method (1930). However, it would seem that oxidation is, in fact, incomplete in some cases, for better recoveries were encountered when a larger excess of sodium peroxide was added. The sample of sodium peroxide used in these investigations was one of high purity from a new sealed original Kalbaum container. The sulphur that is lost is seemingly not further oxidized by alkaline hydrogen dioxide, as sufficient excess of sodium peroxide was present in most cases to cause a marked evolution of oxygen when water was added to the mixture after fusion. The residue left after extraction with acid was in all cases colourless.

The digestiou of fodder and faeces in fuming nitric-perchloric acid mixtures retained variable amounts of sulphur, a constant proportion being lost in each sample examined. This was not altered by doubling the amount of oxidizing reagent and prolonging the time of digestion. The recovery was quantitative, however, when applied to urine, but poor when applied to faeces or to the sample of fodder examined.

Combustion in compressed oxygen gave by far the most consistent and the highest recoveries and this procedure has the advantage of being rapidly and easily carried out.

By the use of the Bomb calorimeter the estimation of total combustible energy, total carbon content and total sulphur content of fodder and excreta of the type discussed above, may be carried out with precision and ease on one sample.

## Conclusions

The above considerations are sufficient to make clear that the recovery of sulphur is at times far from quantitative when certain organic materials are oxidized with reagents which have found wide

application in chemical analysis. Combustion in compressed oxygen confined in a closed bomb is undoubtedly the most precise and accurate means for the destruction of organic matter prior to the estimation of sulphur in such materials as fodder and excreta.

## SUMMARY

Critical estimations of the recovery of sulphur, when materials such as fodder and excreta were subjected to a number of analytical procedures. The only method in this series which resulted in consistently good estimations was one involving oxidation in compressed oxygen confined in an Emerson Calorimeter Bomb.

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*Results of analyses of dried faeces of* $W_3A_{43}A$

| Corrected weight of sample g. | Weight of $BaSO_4$ mg. | | % sulphur in sample | Method |
|---|---|---|---|---|
| 1·8050 | 26·7 | | 0·203 | |
| 1·8009 | 26·6 | | 0·203 | |
| 1·7924 | 26·5 | | 0·203 | |
| 1·8176 | 26·7 | | 0·202 | |
| | | Mean | 0·203 | Bomb (4) |
| 1·8129 | 20·3 | | 0·154 | |
| 1·8600 | 21·1 | | 0·156 | |
| 1·8290 | 20·5 | | 0·154 | |
| 1·8100 | 20·3 | | 0·154 | |
| | | Mean | 0·154 | Benedict-Denis (1) |
| 1·8075 | 26·5 | | 0·183 | |
| 1·7995 | 25·8 | | 0·197 | |
| 1·8089 | 25·9 | | 0·197 | |
| 1·8130 | 26·7 | | 0·201 | |
| | | Mean | 0·195 | Sodium peroxide fusion (2) |
| 1·7998 | 23·5 | | 0·179 | |
| 1·8120 | 24·8 | | 0·187 | |
| 1·8009 | 23·9 | | 0·182 | |
| 1·8005 | 24·8 | | 0·187 | |
| | | Mean | 0·184 | Nitric-perchloric digestion (3) |

*Results of analyses of fodder of* $W_3A_{43}A$

| Corrected weight of sample g. | Weight of $BaSO_4$ mg. | | % sulphur in sample | Method |
|---|---|---|---|---|
| 1·7640 | 23·6 | | 0·185 | |
| 1·7860 | 24·8 | | 0·187 | |
| 1·7500 | 24·2 | | 0·189 | |
| 1·7750 | 24·6 | | 0·189 | |
| | | Mean | 0·187 | Bomb |
| 1·7670 | 24·7 | | 0·191 | |
| 1·7775 | 23·4 | | 0·180 | |
| 1·7800 | 24·4 | | 0·188 | |
| 1·7778 | 23·3 | | 0·180 | |
| | | Mean | 0·185 | Benedict-Denis |
| 1·7805 | 17·6 | | 0·136 | |
| 1·7821 | 17·6 | | 0·136 | |
| 1·7780 | 17·6 | | 0·136 | |
| 1·7790 | 17·8 | | 0·137 | |
| | | Mean | 0·136 | Sodium peroxide fusion with 5 g. $Na_2O$ |
| 1·7720 | 21·8 | | 0·173 | |
| 1·7740 | 21·7 | | 0·170 | |
| 1·7780 | 21·4 | | 0·165 | |
| 1·7749 | 21·7 | | 0·170 | |
| | | Mean | 0·170 | Sodium peroxide fusion with 10 g. $Na_2O$ |

*Results of analyses of fodder of* $W_3A_{43}A$ (*continued*)

| Corrected weight of sample g. | Weight of $BaSO_4$ mg. | % sulphur in sample | Method |
|---|---|---|---|
| 1·7749 | 20·1 | 0·155 | |
| 1·7749 | 21·3 | 0·165 | |
| 1·7770 | 21·5 | 0·166 | |
| 1·7795 | 20·0 | 0·155 | |
| | Mean | 0·160 | Nitric-perchloric digestion (3) |
| 1·7745 | 20·3 | 0·158 | |
| 1·7740 | 21·0 | 0·162 | |
| 1·7740 | 21·4 | 0·165 | |
| 1·7780 | 21·6 | 0·166 | |
| | Mean | 0·163 | Nitric-perchloric digestion. Repeat of (3) |

*Results of analyses of urine of* $W_3A_{43}A$

| Volume represented in sample ml. | Weight of $BaSO_4$ (mg.) in 50 ml. urine | Mg. sulphur per 100 c.c. | Method |
|---|---|---|---|
| 32·09 | 375·0 | 103·0 | |
| 30·65 | 371·8 | 102·0 | |
| 30·27 | 375·0 | 103·0 | |
| 39·00 | 382·0 | 105·0 | |
| | Mean | 103·0 | Bomb (4) |
| 50·0 | 245·7 | 67·6 | |
| 50·0 | 246·9 | 67·8 | |
| 50·0 | 245·9 | 67·6 | |
| 50·0 | 245·4 | 67·4 | |
| | Mean | 67·6 | Benedict-Denis (1) |
| 50·0 | 210·7 | 57·8 | |
| 50·0 | 212·0 | 58·2 | |
| 50·0 | 210·9 | 58·0 | |
| | Mean | 58·2 | Sodium peroxide fusion (2) |
| 50·0 | 375·6 | 103·0 | |
| 50·0 | 373·5 | 102·4 | |
| 50·0 | 373·9 | 102·8 | |
| 50·0 | 373·8 | 102·8 | |
| | Mean | 102·7 | Nitric-perchloric digestion (3) |

# IDENTIFICATION AND MEASUREMENT OF THE COMBUSTIBLE GASES THAT OCCUR IN THE GASEOUS METABOLIC PRODUCTS OF SHEEP

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(With One Text-figure)

MEASUREMENTS of the gaseous metabolism of sheep in this laboratory are conducted by drawing air through a sealed chamber that contains the animal, sampling the effluent air, and analysing the sample for its carbon dioxide increase and its oxygen deficit. As the analysis is dependent upon the measured fraction of nitrogen with rare gases, and particularly in respect of the oxygen deficiency this fraction must be known rather precisely. It is of course assumed that the animal in no way changes the quantities of nitrogen and rare gases passing through the chamber, but the analysis is incomplete if any gases or vapours other than carbon dioxide and water are given off.

It is well known that considerable quantities of methane, and possibly some hydrogen, are produced by ruminants, and some forms of gas analysis apparatus include a device designed to measure the concentration of methane in the sample. The device, as used in the apparatus of Carpenter & Fox (1926), consists of a platinum spiral heated electrically, the carbon dioxide resulting from combustion being measured volumetrically in the ordinary way, together with the $O_2$ consumed. Experience with the device in this laboratory has led to its abandonment and gravimetric methods are now employed instead.

## THE APPARATUS

The apparatus, which has been devised for sampling the chamber gases and analysing the sample for combustible gases that can pass a preliminary purifying chain, is similar to that employing large quantities of chamber gases which has been used by Armsby & Fries (1915) and by earlier workers. The sample is drawn continuously from the chamber and passed through a purifying chain designed essentially to remove carbon

dioxide and water vapour, but removing also any hydrogen sulphide mercaptans, acetone, etc. that may be present. It then passes through a heated combustion tube, through an analysing chain to absorb the

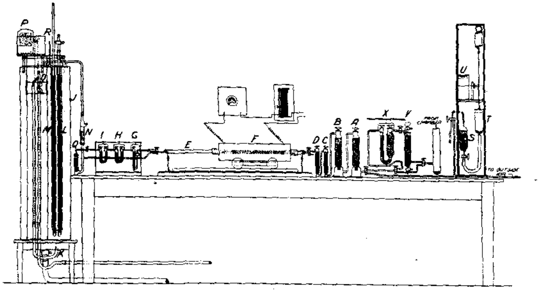

Fig. 1. Arrangement of apparatus for estimation of combustible gases in the gaseous metabolic products of sheep.

products of combustion, and finally into the measuring tank. The apparatus is depicted in Fig. 1. Broadly it consists of six parts:

(1) Purifying chain,
(2) Combustion unit,
(3) Analysing chain,
(4) Sampling and measuring tank,
(5) Test unit (connected with the outside air),
(6) Preformed $CO_2$ estimation unit (connected with the chamber).

(1), (2), (3) and (4) are connected together in that sequence, and (1) may be connected with (5), or with (6), or directly with the chamber. (2) may be connected directly with (4) instead of through (3): this is necessary during preliminary equilibrations.

(1) Consists of four towers, *A*, *B*, *C* and *D*, the first two each of about 600 ml. and the last two each of about 100 ml. empty capacity. The first tower is filled with soda-lime, the second with calcium chloride, the third with "ascarite" (soda-asbestos), and the fourth with phosphorus pentoxide layered in glass-wool.

(2) The silica combustion tube, *E*, is of 1·7 cm. internal and 2·1 cm. external diameter, and 102 cm. long. It is normally filled with cupric oxide wire fragments for a length of 35 cm. commencing 20 cm. from the forward end. An electric furnace, *F*, supported upon a carriage, runs

smoothly along the tube. The furnace was constructed from a silica tube 61 cm. long and of 2·6 cm. internal and 3·2 cm. external diameter, by winding thereon 85 ohms of 24-gauge nichrome wire, and lagging with asbestos sheet to a diameter of 12 cm. It is fed from the 200 V. A.C. supply with a rheostat and ammeter in the circuit. Temperatures at different rheostat settings were measured to within about 20° C. with a thermo-junction pyrometer.

(3) The water analyser, *G*, is one sealed-off limb of a stoppered U tube with a narrow stop-cocked inlet tube fused into it a short distance from the bottom. The limb is filled with alternate layers of phosphorus pentoxide and glass-wool, and exhaustion is readily followed as successive layers of phosphorus pentoxide liquefy.

The carbon dioxide analyser, *H*, is a stoppered U tube filled with "ascarite", and the progressive encroachment of the white exhausted zone upon the fresh grey material is easily observed. It is followed by a guard, *I*, consisting of a small stoppered U tube filled after the manner of the water analyser. The liquefied particles coalesce into drops on the glass-wool and do not cause blockage troubles.

(4) is a cylindrical copper tank, *J*, about 120 cm. in length and 36 cm. in diameter. The bottom end is convex and filled with a metal tap, *K*, for filling purposes. Two vertical glass tubes, *L* and *M*, connect with the tank near the bottom. One, *L*, is connected with the tank again at the top, and the other, *M*, is open to the air above the top. The first gives an index of the tank water level, and the difference in height between the levels in the two tubes is a measure of the pressure relative to atmospheric. Gases from (2) or (3) are drawn over wet filter paper in the humidifier, *N*, and into the tank via a tube in the top, by the simple device of lowering a non-siphoning overflow tube, *O*, which connects with the bottom of the tank. The tube runs vertically on slide-wires and is lowered by chain and cogs ("Meccano" parts) fitted to an electric clock, *P*, the customary rate of discharge being 3 l. per hr. To guard against back diffusion there is an "ascarite" tube, *Q*, following (3). The tank is lagged with cow-hair felt and the rate of flow is such that temperature rises are much too slow to reverse the gas flow.

(5) A pair of cylindrical glass vessels, *S* and *T*, each of about 280 ml. capacity, are connected with rubber tubing at their lower ends and contain about 300 ml. of mercury. One reservoir, *S*, is fixed and the other may be raised at constant rate by chain and cogs fitted to an electric clock, *U*.[1]

[1] The test unit shown in Fig. 1 was constructed from a discarded sampling device described by Lines & Peirce (1931).

(6) This unit is employed to test the sampling, by burning acetone in the chamber. It is frequently employed to provide a gravimetric check upon the volumetric carbon dioxide estimations which must first be reduced to a "pure carbon dioxide" basis by applying the Van der Waals equations for mixtures. It consists of a pumice sulphuric acid drier, *V*, of about 250 ml. empty capacity. From time to time spent acid may be run off from the bottom and fresh trickled in at the top. Leakage of water vapour is of the order of only 0·3 mg. per 70 l. and a phosphorus pentoxide backing is unnecessary. Following the drier is a larger U tube, *X*, containing "ascarite" in the first limb and phosphorus pentoxide layered in glass-wool in the second.

The only delicate operations are the weighings of the analysing tubes described in (3). Immediately before weighing, a tube is thoroughly wiped and then allowed to remain on the balance for not less than 15 min. and with the inlet open. The air within the balance case is kept dry. This, or some equally satisfactory precaution against the effects of varying atmospheric humidity upon large glass surfaces, is necessary. Individual weighings are easily made to within 0·1 mg. constant for more than 3 min. and are frequently subject to small buoyancy corrections for a change in air density between the beginning and end of an experiment.

## BLANKS

Several factors may contribute towards blanks and an effort was made to find which of these may be operative during an estimation. A series of tests was run upon outside air drawn from the vicinity of the respiration chamber inlet. The results may be summarized:

(*a*) With the combustion tube empty or containing cupric oxide, and the furnace at 700–850° C., the blanks average 0·75 mg. $H_2O$ and 0·6 mg. $CO_2$.

(*b*) With cupric oxide in the tube and the furnace at 540–580° C. the blanks average 0·65 mg. $H_2O$ and 0·45 mg. $CO_2$.

(*c*) With an empty tube and cold furnace the blanks average 0·35 mg. $H_2O$ and 0·3 mg. $CO_2$.

In every case the amount of air used was approximately 67 l. on a dry N.T.P. basis.

From the results of combustion tests with methane and hydrogen (see below) it is concluded that in (*a*) any hydrogen or methane, or both, in the outside air would be completely burned, whereas in (*b*) the hydrogen only would be burned. The evidence is therefore that there are about 4 parts of hydrogen and 2 of methane per million of outside air, neglecting

the possibility that nitrogen oxides are produced during combustion. Although the comparatively large weighing errors make these figures uncertain, their smallness and reasonable constancy over a period of about 4 months are reassuring. The hydrogen figure is only about 2 % of Gautier's (1898) and 15 % of Rayleigh's (1902), but is in agreement with Claude's (1909) estimate of less than 10 parts per million.

Although the glass tubes are always well butted together, the greater part of the blanks in (*c*) must be ascribed to diffusion through the rubber junctions. The dead space of the analyser side-tubes and lead from the combustion tube is insufficient to account for more than a small fraction of them, even supposing this space to be filled with room air but neglecting adsorption of carbon dioxide and water vapour by its walls; and from the appearance of the final purifiers and the fact that addition of carbon dioxide and water vapour to the ingoing air is without effect on the blanks, there can be no appreciable leakage through the purifiers.

The methane content of the effluent chamber gases is found to be roughly 1 part in 2000, giving on combustion a like concentration of carbon dioxide and twice that concentration of water vapour. This is approximately the carbon dioxide content of room air but only a fraction of water vapour content. The appropriate blanks per 67 l. N.T.P. of dry chamber gases with the furnace above 700° C. are taken to be:

$$0{\cdot}4+0{\cdot}3=0{\cdot}7 \text{ mg. } H_2O, \text{ and } 0{\cdot}3+0{\cdot}1=0{\cdot}4 \text{ mg. } CO_2.$$[1]

## Combustion tests with methane and hydrogen

Methane was prepared from the Grignard reagent $\left(Mg\langle{}^{I}_{CH_3}\right)$ by the method of Tissier & Grignard (1901). Only about 10 % of the gas was collected (200 ml.), the rest being used to flush out the apparatus at reduced pressure. The gas was freed from traces of ether and water vapour by passing through three sulphuric acid bubblers, the last bubbler being kept below −5° C., and was then stored under pressure in a gas-holder. The hydrogen was electrolytic tank hydrogen. Both gases were assumed to be pure.

Quantities of the gases were measured in a small gas burette and transferred to the fixed reservoir of the test unit. Here they were mixed with about 200 ml. of carbon dioxide and slowly fed into the stream of

[1] After an accident, the furnace was replaced by one of similar dimensions, in which the insulation was provided by Kieselghur contained in a brass cover. Currents induced in the cover were too small to warm it. At the same time, the combustion tube was replaced, and whilst the water blank above 700° C. remained unaltered, the carbon dioxide blank was increased to 0·6 mg.

outside air that had been saturated with water vapour and was passing through the system. About 15 l. of air were used for the purpose, and after complete discharge of the gases from the test unit a further 5 l. were drawn through for scavenging. The results are given in Table I.

Table I

| Test no. ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| Furnace temp. ° C. | 500 | 775 | 540 | 775 | 635 | 685 | 500 | 540 | 720 |
| $H_2$ added ml. N.T.P. | — | — | 32·32 | 27·06 | — | — | 7·47 | 7·86 | 8·04 |
| $CH_4$ added ml. N.T.P. | 18·56 | 18·40 | — | — | 16·16 | 15·58 | 16·53 | 16·49 | 17·45 |
| $H_2O$ mg.* theory | 29·9 | 29·6 | 26·0 | 21·8 | 26·0 | 25·1 | 32·6 | 32·9 | 34·6 |
| $CO_2$ mg.* theory | 36·5 | 36·2 | — | — | 31·8 | 30·7 | 32·5 | 32·4 | 34·3 |
| $H_2O$ mg. weighed | 0·5 | 29·6 | 26·4 | 22·0 | 9·6 | 25·3 | 5·2 | 6·5 | 34·8 |
| $CO_2$ mg. weighed | 0·3 | 36·5 | 0·3 | 0·3 | 12·0 | 30·6 | 0·4 | 0·6 | 34·4 |
| $H_2O$ blank mg. calc. | — | 0·2 | 0·2 | 0·2 | — | 0·2 | — | — | 0·2 |
| $CO_2$ blank mg. calc. | — | 0·0 | 0·1 | 0·2 | — | 0·0 | — | — | 0·0 |
| Error $H_2O$ est. mg. | — | −0·2 | 0·2 | 0·0 | — | 0·0 | — | — | 0·0 |
| Error $CO_2$ est. mg. | — | 0·3 | 0·2 | 0·1 | — | −0·1 | — | — | 0·1 |

* For complete combustion.

It will be noticed that the combustion of hydrogen is complete above 540° C., and of methane, above 685° C. Tests 1, 7 and 8 suggest that there is slight combustion of methane even at 500° C. whilst the combustion of hydrogen is not quite complete there, but that a moderately good separation is possible at 540° C. For those tests in which combustion was obviously complete (2, 3, 4, 6 and 9), appropriate blanks have been calculated from the work discussed in the preceding section, and subtracted from the difference "weighed" minus "theory" to give the errors of estimation shown in the final two columns. The errors are not uniformly zero and are actually positive in the aggregate, but almost every one lies within the error of weighing and the tests are considered satisfactory. In discussing the blanks in the preceding section, the possible formation of nitrogen oxides was disregarded. Notes that bear on this matter are found in Mellor's *Comprehensive Treatise on Inorganic and Theoretical Chemistry*, **8** (1928), and from them one might anticipate slight formation particularly when burning hydrogen. However, the tests indicate no serious interference from this source.

### COMBUSTIBLE GASES PRODUCED BY THE SHEEP

The volume of gas entering the tank is obtained from the tank scale. It is reduced to a dry N.T.P. basis, and corrections are made for the carbon dioxide removed by the purifiers or estimation unit (0·5–1 %), and for the combustible gas burned together with the oxygen thereby consumed (0·1–0·2 %). The volume of effluent chamber gases varies between 34,000 and 38,000 l. N.T.P.

Table II

| Experiment no. ... | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|
| Tank volume corrd. litres dry N.T.P. | 66·3 | 66·8 | 67·2 | 67·3 | 66·8 | 66·4 | 67·0 | 65·9 |
| $H_2O$ mg. weighed minus blank, 0·7 mg. | 57·1 | 50·3 | 48·8 | 48·8 | 60·3 | 46·6 | 62·9 | 61·5 |
| $CO_2$ mg. weighed minus blank, 0·4 mg. | 69·8 | 61·7 | 59·6 | 59·7 | 73·6 | 56·7 | 77·2 | 75·0 |
| Ratio of H atoms to C atoms | 4·00 | 3·98 | 4·00 | 3·99 | 4·00 | 4·02 | 3·99 | 4·01 |
| Methane ml. N.T.P. calc. from the $CO_2$ | 45·5 | 31·4 | 30·3 | 30·3 | 37·4 | 28·8 | 39·2 | 38·1 |

In Table II are given some data of preliminary trials with sheep in the chamber and the furnace running at 720° C. The fifth row gives the number of hydrogen to carbon atoms in the combustible gas. The number averages 3·999 with a standard deviation of 0·012, and suggests that the gas is extremely pure methane. That the gas is not some other hydrocarbon with a definite proportion of hydrogen has been proved fairly well by running the furnace at 540° C., when, after subtracting appropriate blanks, the gains in the water and carbon dioxide analysers correspond to within the limits of accuracy of weighing with the combustion of about 0·7 instead of about 35 ml. N.T.P. of methane.

The sheep used in these preliminary tests received a ration of 2 parts of wheat straw chaff and 1 part of chaffed lucerne hay. The heat of combustion of the methane produced (some 18 l. N.T.P. per day) is about one-eighth of the difference between the heat of combustion of the intake and that of the faeces plus urine. The result is in reasonable agreement with earlier published work on ruminants.

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# ON ALIMENTARY ACETONURIA AND KETONURIA IN DAIRY CATTLE INDUCED BY FEEDING GRASS SILAGE OF THE BUTYRIC ACID TYPE

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For many years ketone bodies (beta-oxybutyric acid, $CH_3.CHOH.CH_2.COOH$; acetoacetic acid, $CH_3.CO.CH_2.COOH$; and acetone, $CH_3.CO.CH_3$) have played a prominent role in the pathology of men and of some domestic animals. These bodies appear to be mainly formed in the liver (cf. Mirsky, 1936) by the incomplete oxidation of the fatty acids.

In some diseases these bodies are formed in large amounts; in man they are encountered especially in diabetes mellitus, and in the cyclic vomiting of children. In the ruminants (cattle, sheep) the disease acetonemia (acetonuria) is well known. In cattle this disease develops most frequently some weeks after parturition (also called post-parturient dyspepsia), in sheep during pregnancy (also called pregnancy disease). Evidently the nomenclature is ambiguous, because by the same names (acetonuria, acetonemia) a symptom only is sometimes indicated, whilst in other cases a distinct disease is denoted.

In recent years several authors are of the opinion that ketonuria often occurs in apparently healthy dairy cows. We have ourselves observed at times a faint acetone reaction (Legal) in the urine of grazing cattle. Much more frequently the symptom is stated during stabulation (Boddie, 1933, 1935).

It is not perfectly clear whether the symptom in the cases just mentioned is without any significance for the general health of the animals; some investigators believe it to be the result of a metabolism derangement that stands principally on one line with the disease acetonemia, differing from the latter only in intensity. Boddie (1933, 1935) suggests that apart from those cows which were manifestly ill, there must be many whose health and resistance to other disease were undermined by the existence, for a substantial part of the year, of a degree of ketosis insufficient in itself to produce clinical signs but obviously sufficient to

have a very considerable effect on the tissues, particularly perhaps the parenchymatous organs.

Some authors believe that the acetonuria (with or without clinical signs) has an alimentary origin. Roderick *et al.* (1937) have even succeeded in producing in well-fed ewes the whole of the usual pathological processes which are regularly recognized in clinical pregnancy disease, by the simple expedient of starvation. Milder forms of this pathological condition were produced in lambs. Yet, the problem is far from being solved.

## Experimental

In the present paper we report upon a form of ketonuria, without clinical signs, that is undoubtedly of alimentary origin, which results from feeding cattle with a large amount of grass silage of the butyric acid type. A demonstration of our observation, which has been substantiated in further trials not mentioned here, is given in the following experiment.

Eight milch cows (date of parturition at the latest 20 October 1937; mean milk production 11 kg. per cow per diem) were divided up into two groups, nearly equivalent in regard to live weight, age, date of calving, milk and milk-fat production. In group I the daily ration per cow consisted of 30 kg. grass silage (*p*H 5·2, butyric acid content 1·7–2·0 %), 3 kg. grass hay and an average of 2·3 kg. concentrates (equal parts of dried sugar-beet pulp, palm-kernel meal, maize meal, barley meal, earthnut-cake meal, and linseed meal, plus 1 % common salt).

Group II got no silage, its ration consisting of 13 kg. grass hay and an average of 2·6 kg. of the same concentrate mixture as in group I.

The urine had the following composition (Table I):

Table I

| Group I (silage) | | | | Group II (hay) | | | |
|---|---|---|---|---|---|---|---|
| Cow no. | Acetone (Legal) | Ketone bodies (g./l.) | $NH_3$ (milli-equivs./l.)* | Cow no. | Acetone (Legal) | Ketone bodies (g./l.) | $NH_3$ (milli-equivs./l.)* |
| 1 | + | 1·04 | 3·7 | 15 | – | 0·23 | 3·0 |
| 10 | + | 0·46 | 2·5 | 18 | – | 0·26 | 1·5 |
| 33 | + | 0·83 | 6·0 | 22 | – | 0·34 | 2·4 |
| 50 | – | 0·32 | 3·0 | 58 | – | 0·30 | 2·0 |
| | Mean | 0·66 | 3·8 | | Mean | 0·28 | 2·2 |

$* = \frac{\text{mg./l.}}{17}$.

In this table it is shown that the acetone test of Legal[1] was distinctly positive in three animals of the silage group whilst it was negative in the

[1] Still more delicate is the test of Rothera; in both acetoacetic acid also reacts (see Engfeldt, *Biochem. Z.* **159** (1925), 257).

hay group. The content of total ketone bodies (determined by Van Slyke's[1] method) was considerably higher in the silage group than in the hay group in which the amount was about normal. Finally, in the silage group the ammonia content was somewhat higher, although within normal limits. From this latter fact we conclude that an acidosis was altogether out of the question (Brouwer & Dijkstra, 1936) since, indeed, the urine remained alkaline.

The same day, 21 January, the rations were exchanged: Group I then received 13 kg. hay, Group II 30 kg. silage + 3 kg. hay, the concentrates remaining the same for both groups. Immediately, 22 January, the acetone reaction in the former silage group practically disappeared. More extensive urine analyses were performed on 24 and 28 January with results shown in Table II.

Table II

| Group I (hay) | | | | | Group II (silage) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Cow no. | Acetone (Legal) | Ketone bodies (g./l.) | $NH_3$ (milli-equivs./l.)* | pH | Cow no. | Acetone (Legal) | Ketone bodies (g./l.) | $NH_3$ (milli-equivs./l.)* | pH |
| | | | | 24 January | | | | | |
| 1 | – | 0·28 | 2·2 | 8·35 | 15 | + | 3·41 | 6·2 | 8·00 |
| 10 | – | 0·24 | 4·0 | 7·90 | 18 | + | 2·02 | 6·5 | 8·15 |
| 33 | – | 0·28 | 3·0 | 8·05 | 22 | + | 4·56 | 8·0 | 7·50 |
| 50 | – | 0·32 | 2·0 | 8·30 | 58 | + | 2·71 | 6·0 | 7·50 |
| | Mean | 0·28 | 2·8 | 8·15 | | | 3·18 | 6·7 | 7·79 |
| | | | | 28 January | | | | | |
| 1 | – | 0·17 | 3·4 | 8·30 | 15 | + | 1·89 | 4·3 | 8·10 |
| 10 | – | 0·19 | 5·2 | 7·95 | 18 | + | 0·82 | 4·5 | 8·25 |
| 33 | – | 0·37 | 7·0 | 8·05 | 22 | + | 1·14 | 5·5 | 7·80 |
| 50 | – | 0·24 | 2·5 | 8·30 | 58 | + | 0·98 | 6·2 | 7·85 |
| | Mean | 0·24 | 4·5 | 8·15 | | | 1·21 | 5·1 | 8·00 |

$* = \frac{\text{mg./l.}}{17}$.

It will be seen that now in Group II the acetone reaction was positive and that the content of total ketone bodies increased, the latter being distinctly greater on 24 January than on 28 January. Perhaps, in the meantime, the animals became accustomed to the silage feeding. Clinically, they never seemed to be ill.

Again, there was no question of acidosis, the urine remaining alkaline and the ammonia content low. To be sure there was in this respect a small difference between the two groups (in the silage group somewhat

[1] Peters, Van Slyke, *Quant. clin. Chem.* II (1932). If the differences are small, a blank determination should be performed (Peters, Van Slyke, p. 628), because the urine always contains some substance that gives the same precipitation reaction. Usually this blank determination is omitted, and we acted in the same way after it had in some experiments been shown that the results remained essentially the same.

higher ammonia content and somewhat lower *p*H), but these small differences have no practical meaning. Much more important are the positive acetone reaction and the increased content of total ketone bodies in the silage group.

## Discussion

As to the cause of the acetonuria in these cases; *a priori*, we considered it practically certain that it was due to the butyric acid in the silage, for it is generally known that in incomplete oxidation butyric acid gives rise to ketone bodies, because, in metabolism, it behaves in many respects like the other fatty acids. It is regularly present in butter, but cannot be deposited in the body fat (Eckstein, 1929) nor be converted into depositable fatty acids (Rittenberg *et al.*, 1937) nor into glucose or glycogen (Deuel *et al.*, 1935–6). An excessive amount, therefore, must be burned by beta-oxidation and may yield in this way a certain amount of ketone bodies if the oxidation is incomplete; thus, the butyric acid is "ketogenic". As a matter of fact, this acid is more ketogenic than the natural fats, the glycerol component of the latter according to many authors being "antiketogenic" (Deuel *et al.* 1935–6).

Lactic acid, on the contrary, in metabolism may, like glycerol, endure transformation into glucose and glycogen; therefore, it also is an "antiketogenic" substance.

That the ingested amounts of butyric acid may be really large results from the fact that in some cases we found more than 2 % of this substance in butyric acid silages (Brouwer, 1937), that is more than 200 g. in 10 kg. silage. Some authors have found ketone bodies also in silage. In our cases, however, amounts of these substances were at most very small.

Later, we had at our disposal a grass silage of the *lactic acid type*, containing no butyric acid at all. The silage had been prepared in a concrete silo with tight bottom; 2 % sugar had been added in the filling, the *p*H was approximately 4·0, the acetic acid and lactic acid contents were 0·5 and 2·1–2·2 % respectively, and the juice gave no acetone reaction (Rothera). On feeding each of four cows daily 35 kg. of this silage the acetone reaction in the urine of three of them was negative. Nevertheless one cow developed a distinct positive acetone reaction (by Legal and by Rothera).

We repeated the experiment with this cow several times. On the days on which only hay was fed the acetone reaction was always negative, whereas on feeding the lactic acid silage the reaction without exception turned positive. We even succeeded in preparing from the urine dibenzal-

acetone (Abderhalden, 1931) (M.P. 113° C.), which proved definitely that acetone or acetoacetic acid was present.

We soon observed a close relation between the acetone reaction and the time at which the silage was fed. In giving 12–15 kg. at 6.45 a.m. and again 12–15 kg. at 7.45 a.m. the acetone reaction was negative until about 8 a.m., then turned positive and was again negative at about 1 p.m., except when the silage was not eaten at once.

As the quantity of ketone bodies in the urine was rather small (about 0·5 g. per l.), we thought it probable that some butyric acid in the silage might have been undetected by the method we used (Wiegner). We had, however, an opportunity of feeding the same cow on succeeding days with the above-mentioned lactic acid silage and a butyric acid grass silage which contained 1·3–1·8% butyric acid. Both kinds of silage were fed in the morning only at 6.45 and 7.45 and in the afternoon the animal received grass hay. On 26 and on 29 April the cow got 30 kg. of the lactic acid silage which was devoid of butyric acid, and on 27 and 28 April 23·1 and 25·6 kg. of the butyric acid silage containing 310 and 458 g. butyric acid. The silages were used in such quantities that the amounts of dry substance fed were about the same.

Each day the portions of urine voided between 6 a.m. and ± 5 p.m. were collected and analysed separately. We were much surprised to find the maximum concentration of ketone bodies each day almost the same. In the case of lactic acid silage it was 0·50 and 0·60 g./l. and for the butyric acid silage 0·54 and 0·74 g./l. Nor were the total quantities of ketone bodies voided much different.

As this experiment was made with only one cow, we do not know, of course, how far this animal is exceptional. Yet the experiment with this very closely observed animal tells us that the question of ketonuria in silage feeding may be more complex than we originally supposed.

Our findings on ketonuria in silage feeding have revealed to us only *one* cause of acetonuria in cattle. Without any doubt there must be other causes, for it is quite certain that cases of clinical and non-clinical acetonuria in many instances develop without feeding any silage at all. Nevertheless, the thought may occur whether there exists some relation with our observations, for it might be possible that in these instances the ketogenic substances are not formed by fermentation in a silo, but by an abnormal fermentation of the food in the intestinal tract, e.g. in the paunch. Indeed, as to the disease acetonuria, this hypothesis is not a new one. Wester (1935) e.g. considers the enterogenic origin of the acetonuria not

completely excluded. Some observations of Koffman (1937) are also worthy of note. This investigator, however, tried to prove that acetone is formed already in the paunch from the carbohydrates of the food. However, in some cases of clinical acetonuria without silage feeding, we did not succeed in detecting an abnormal fermentation in the paunch; yet, in our opinion, the question has not been settled and deserves further investigation.


## Summary[1]

Attention has been directed to the occurrence of acetonuria in apparently healthy and in diseased cattle.

It has been shown possible to cause an *alimentary acetonuria* in milch cows experimentally by feeding large quantities of grass silage of the butyric acid type. The reaction of the urine remained alkaline and the ammonia content was within a normal range, showing that there was no real acidosis. The animals seemed by no means to be ill.

As to the cause of the acetonuria, *a priori*, there seemed to be little doubt that in these cases the ketones resulted, in the animal body, from the incomplete oxidation of the butyric acid of the grass silage. Yet, further experiments with a lactic acid silage containing no butyric acid at all indicated that the question may be more complex.


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